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Microbial Load and Resistance Pattern of Bacteria Organisms in the Gastrointestinal Tract of Household Rat

J.E. Egbagba[1*], U.B. Owhe-Ureghe[2], M.A. Alex-Wele[3], S.D. Lawson[4] and L.A. Orutugu[5]

[1]Department of Medical Microbiology, Federal Medical Centre Yenagoa, Nigeria
[2]Department of Microbiology, Faculty of Science, Delta State University, Abraka, Nigeria
[3]Deartment of Medical Microbiology, University of Port Harcourt Teaching Hospital Port Harcout, Nigeria
[4]Department of Medical Microbiology and Parasitology, Rivers State University, Port Harcourt, Nigeria
[5]Department of Medical Microbiology and Parasitology, Niger Delta University, Wilberforce Island, Amassoma, Nigeria
**Corresponding author*

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A B S T R A C T

Household rats are known agents of human diseases worldwide. They are vehicles of transmission of bacteria, viral, fungal and protozoal disease to man. Rats and human guts with similarities are colonized with normal flora that can harbour multidrug-resistant organisms transmissible to man. This study investigates the microbial load of household rats and their resistance micro-organisms which are transmissible to man. This knowledge is useful for household rat disease burden management in our environment and in healthcare setting. A cross-sectional descriptive and analysis of 200 trapped rat collected from 100 household in Abraka, Nigeria after consent were obtained. The trapped rats were killed, dissected and swab taken from each throat, small and large intestine respectively for analysis of bacteria and fungi load (Using serial, dilution and viable count method). Other biochemical tests for bacteria and fungi analysis were done according to standard Microbiology methods. The results obtained shows more bacteria load in small intestine of household rats compared to throat and large intestines. *Bacteriodes fragilis* and *Escherichia coli* were the predominant bacteria obtained, while *Candida albicans* and other Candida species were the most abundant fungi seen. More than two-third of the bacteria isolates were resistant to commonly used antibiotics such as Ampicillin, Penicillin, Trimetoprin-suphamethoxazole, and Cefriazone. Households rats in our study indicate a high burden of bacteria and fungi load with increased bacteria resistance. Reporting of this zoonotic disease associated with these organisms, proper diagnosis and management are required to mitigate this potential source burden of these disease causing agents.

Introduction

The house mouse (*Mus musculus*) infestation has been a worldwide problem for ages and are known reservoirs of a number of human diseases.[1]

Rodents are known agents of many human bacterial, viral, protozoa and fungal diseases[1,2,3]. They have been linked with the risks and transmission of diseases such as Salmonellosis, Campylobacteriosis, Gastro-enteritis, Candidiasis, rat bite fever, leptospirosis, plague, and murine typhus epidemic[3,4]. Additionally the presence of household rats have been associated with multidrug resistant organisms that are likely transmittable to man[4,3,5].

General agreement exist about the similarities of rat and human gut normal flora. These consist of facultative bacteria such as *Streptococcus, Staphylococcus, Escherichia coli* and other Entero bacteriacae, however, the anaerobic bacteria predominate especially in the large gut with *bacteriodes fragilis, bifido bacterium* and *Lactobacilli.*[6,7,8]. Similarly, the predominant fungi which colonize the gut includes *Candida albican*, other candida species, *Malassezia furfur* and the filamentous fungus, *Cladosporium.*[9,10].

Although various researches have looked at rat gut microbial composition in relation to human disease transmission, however few have looked at its antimicrobial resistant organism that are possibly transmissible to man.

There are scanty studies about rat zoonotic diseases in our environment and most zoonotic diseases are under reported. This poses a health risk to the populace and difficulty in disease management. [11] This article therefore set out to determine the microbial load of household rats and its antibiotic resistance organism that are transferable to man. The outcome of this study could be useful for furtherance of rat zoonotic diseases management in our environment and in healthcare setting.

Materials and Methods

Study Design and sampling

This cross-sectional analytical study was carried out in Abraka community in Ethiope East local Government Area, Delta state, Nigeria. Abraka is a town in Delta central senatorial district of Delta State with a population estimate of 32,069 and home to the famous Delta State University, Abraka Nigeria. The populace apart from the University Staffs and students are chiefly agrarian. Analysis were done between august to December 2020. Consent was obtained from the community leaders and the heard of each household where rat were obtained with the aid of mouse trap.

Sampling technique

A total of 100 mouse were obtain from 20 households. Rats were trapped using mouse glue board, measuring 30 cm in length and 20 cm in width. After thorough visual inspection of the room to identify rodents' tracks, droppings, the glue board was placed on their path. Pieces of crayfish or smoked fish were placed in the middle of the board as bait. The glue board was left overnight and trapped rodents were collected alive and taken to the laboratory for identification and microbiological analysis. The study was approved by the Delta State University Ethical review Board.

Preparation and Analysis of samples

Each of the rat samples were picked from the trap using forceps. The skin was swabbed

using a sterile swab stick. The rats were dissected using a dissecting kit to obtain the large and small intestine. The throat, small and large intestines was also swabbed using a sterile swab stick and transferred respectively into sterile mortar and then crushed using a sterile pestle to homogenize the intestine. The homogenate (Ig) was taken into a sterile test tube for serial dilution, enumeration and plating protocol.[12] The sample (1g) were collected into a test tube containing 9mls of distilled water. Serial dilutions was then carried out on 5 test tubes and the 10 and 1O dilution of both dilutions were collected and used for pour plate. The sterile swabs were then streaked plated onto sterilized Nutrient agar, McConkey agar and Saboroud Dextrose agar. Inoculated plates incubated at 37°C for 18-24hrs. After incubation in both aerobic and conditions, the colonies were counted using 'Viable Plate count Method' as enumerated by Eby Basiri (Microbiology Biol. 275)[13].

Biochemical Tests

The biochemical tests carried out as described in Microbiology laboratory manual (2014)[14]. The tests includes gram stain, motility, citrate utilization test, Oxidase, indole, catalase, triple sugar iron agar (TSI), Bile esculin agar, and sugar fermentation test (lactose, glucose, sucrose, xylose, galactose, maltose, D-sorbitol, D-mannitol mannose). Antibiotic sensitivity tests were performed using the standard diffusion technique(CLSI 2017[15]. Most commonly used antibiotics discs were used for sensitivity testing and includes Ampicilli, Penicilin, amoxicillin, Trimetoprin-Sulphamethoxazole, Gentamycin, Ceftriazone, Imipenem and Vancomycin.

Results and Discussion

We set out to determine the various bacterial and fungi organisms in the gastrointestinal tracts of household mice as well as their antibiotic resistance pattern. The microbial load from different parts of the gut, shows that the small intestines of our sampled rats has more bacterial and fungal load in comparison to the throat and large intestines (Table 1).

Figure.1, shows the various bacteria organisms and relative percentage prevalence obtained from cultures after 24 hours of incubation. It is evident that *Bacteroides fragilis* and *Escherichia coli* has the highest percentage prevalence of 28% and 15% respectively. This is followed by *Salmonella typhimurium* (13%) and *Staphylococcus aureus* (9.6%).

As shown in figure 2, the predominant fungi species isolated includes *Candida albican* (35.3%) and other candida species (26.6%).

Table 2 show the relative number of each bacteria isolates, and their various percentage resistance to each antibiotics used in sensitivity testing. A total of 375 bacteria organisms were isolated. Of this, a total of 327 and 224 bacteria isolates were tested against ampicillin and tetracycline discs respectively. Notably, all 12 species isolated bacteria from culture are nearly 100% percent resistant to Ampicillin and penicillin(Table 2). More than two-third of the isolates were also resistant to Tetracycline, 224 (77.9%), Amoxicillin-Clavulanic acid, 128 (71.5%), Trimetoprin-Sulphamethoxazole, 207 (60.2%) and Ceftriaxone 343 (60.1%). However, most isolates teste were sensitive to Vancomycin 38 (5.8%), Clindamycin 96 (21.3%) and Amikacin 271 (45.5%).

In recent years, there exist a growing concern about the increase in zoonotic diseases transmission and emergence of resistance organism between house hold rats and human[2]. This indicate risk associated frequent contact between human, household and other human pets.

The result obtained from this investigation shows an abundant number of bacteria and fungi organisms in the gut of household rats that are possibly transmissible to man. It also shows that over two-third of the bacteria organisms isolated were resistant to commonly used antibiotics such as Ampicillin, Penicillin, Tetracycline, Trimethoprim-Suphamethoxazole and Ceftriaxone. The gut microbial population (Table 1) consists of bacteria and fungi which may be symbiotic, neutral or harmful to the host.[16]

These microbiota are important for the host immune response to diseases and maintenance of healthy state [16]. Possible mechanism for abundant microbial load includes genetics, diets and feeding habits.[17],[18] The abundant microbes in our results is consistent with report of Li Wen *et al.*, (2017), which shows that gut microbes increases significantly from the stomach, jejunum to large intestines. The large gut harbours up to 1000 different bacterial and additional 1 x 10^{14} different organisms.[16]

The largest gut bacteria flora in this study were *bacteriodes fragilis* followed by *Escerichia coli* and *Salmonella typhimurium* respectively. Similar results have been reported by Geraldine O. Canny *et al.*, (2008) who reported *bacteriodes fragilis* as the largest gut flora. This result is in contrast to result presented by Tomotari Mitsuoka (1992), who reported that lactobacilli actually predominate in rat and human intestines[7,8]. Possible speculation to differences in gut microbes could be due to the breeding, diets and environmental condition of the host[19,1,17].

Fungi isolates which dominated our study were *candida albicans*, and other candida species. This shows that fungi are also part of the normal flora of household rats as in human. These results are in agreement with previous studies which shows that candida and other fungi species are part of the rat gut ubiquitous microbiota and also serves as either commensals, pathogens or opportunistic organisms[20,21,22].

Perhaps, the most clinically relevant finding in this study is the carriage of antibiotic resistant organisms by these rats (Table 2). As a result of these carriage, transmission of resistant organisms to humans are highly probable through a variety of routes. These routes includes direct contact, the food chain, (where rats are part of the household diets or come into direct contact with food) and contact with direct surfaces.[2],[23],[24] This can lead to the advent of difficult-to-manage diseases[24,25].

Household rats have a closer relationship with humans and they have sought a niche role in human-occupied structures such as houses, classrooms, and restaurants. This, along with other urban rats, are found to host antimicrobial-resistant bacteria such as *E. coli* and *S. aureus*, which can be readily spread to humans by (1) direct or near interaction and (2) ingestion of utensils and fruit. Non-hygienic food handling will escalate this.[11]

Table.1 Microbial load from different parts of rats

Part	Microbial load (cfu/gX10^7)	
	Bacteria	**Fungi**
Throat	2.60	1.96
Small intestine	4.89	2.90
Large intestine	3.73	1.20

Table.2 Antimicrobial resistance pattern of bacteria isolates from cultured plates in the study

Antibiotics	**Bacteria isolates**												
No of isolates Tested	***Bacillus subtillis***	***Bacteides fragilis***	***Salmonella* typhymuium**	***Sudomones aeruginosa***	***Yersinia pestis***	***Pseudomonas***	***Kledsielle***	***Ecscheelichie coli***	***Enterodacter aerogenes***	***Staphylococcusaures***	***Coagulatesnegative staph***	***Samoelladysenteriae***	**Total**
Ampicillin	**15(100)**	**27 N(%)**	**17 N(%)**	**16 N(%)**	**9 N(%)**	**7 N(%)**	**7 N(%)**	**13 N(%)**	**9 N(%)**	**2 N(%)**	**15 N(%)**	**8 N(%)**	**2 N(%)**	**375 N(%)**
		103(100)	48(100)	30(100)	0(0)	11(100)	7(100)	55(100)	5(100		35(100)	9(100)	9(100)
	15(100)	**0(0)**	**48(98.0)**	**30(80.0)**	**0(0)**	**11(10.8)**	**7(20.6)**	**55(100)**	**5(100**		**35(80.0)**	**9(100)**	**9(90.0)**
Penicilin	**15(100)**	**100(80)**	**48(100)**			**11(100)**	**7(98.8)**	**55(100)**	**5(100)**		**35(80.0)**	**9(100)**	**9(100)**
Sulphamethaxazole-Trimethoprim Amixillin	**0((0)**	**0(0)**	**48(20.8)**	**30(45.0)**	**0(0)**	**11(20.6)**	**7(70.3)**	**55(65.5)**	**5(100)**		**33(40.6)**	**9(80.0)**	**9(100)**
Clavulanic acid	**0(0)**	**0(0)**	**0(0)**	**25(60.5)**	**0(0)**	**0(0)**	**0(0)**	**50(85.6)**	**5(80.5)**		**31(60.5)**	**9(60.8)**	**8(80.8)**
Cefoxitin	**15(75.5)**	**100(70)**	**0(0)**	**29(30.8)**	**0(0)**	**11(45.0)**	**7(0)**	**53(70.0)**	**5(68.0)**		**35(35.5)**	**9(40.6)**	**8(70.6)**
Ceftriaxone	**12(80.1)**	**102(70.)**	**45(25.0)**	**27(35.7)**	**26(70.7)**	**11(52,1)**	**7(0)**	**55(86.0)**	**5(100)**		**35(80.8)**	**9(80.5)**	**9(40.9**
Chloramphenicol	**15(30.4)**	**98(5.0)**	**48(15.0)**	**25(75.8)**	**0(0)**	**0(0)**	**7(1.7)**	**50(68.9)**	**5(89.6)**		**30(60.6)**	**9(40.0)**	**7(30.9)**
Erythromycin	**0(0.0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**11(60.0)**	**5(60.8)**	**0(0)**	**5(70.4)**		**30(30.5)**	**8(100)**	**7(80.5)**
Ciprofloxacin	**0(0)**	**0(0)**	**48(15.0)**	**25(5.6)**	**26(70.0)**	**11(3.0)**	**7(0)**	**55(42.9)**	**5(65.5)**		**35(50.9)**	**9(50.0**	**7(30.7)**
Imipenem	**0(0)**	**0(0)**	**48(15.0)**	**25(0)**	**26(0)**	**11(0)**	**7(0)**	**35(20.5)**	**5(70.0)**		**30(80.8)**	**6(80.5)**	**6(0)**
Nalidixic Acid	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**9(20.8)**	**0(0)**	**30(70.4)**	**0(0)**		**0(0)**	**0(0)**	**0(0)**
Gentamicin	**15(80.0)**	**103 (25.9)**	**40 (70.0)**	**25(24.9)**	**25(45.9)**	**11(40.2)**	**7(57.5)**	**53(60.0**	**0(0)**		**30(60.5)**	**6(0)**	**7(70.8)**
Vancomycin	**2(8.5)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**		**30(3.0)**	**6(0)**	**0(0)**
Nitrofurantoin	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**5(70.1)**	**0(0)**	**0(0)**		**0(0)**	**0(0)**	**0(0)**
Metronidazole	**15(2.0)**	**103(0)**	**0(0)**	**0(0)**	**(0)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**		**0(0)**	**0(0)**	**0(0)**
Amikacin	**15(5.0)**	**100 (67.0)**	**40(45.5)**	**25(6.0)**	**26(65.6)**	**0(0)**	**0(0)**	**0(0)**	**35(89.5)**		**30(40,4)**	**0(0)**	**0(0)**
Clindamycin	**0(0)**	**0(0)**	**0(0)**	**25(30.3)**	**0(0)**	**0(0)**	**0(0)**	**0(0)**	**30(55.0)**		**35(30.5)**	**6(0)**	**0(0)**

Fig.1 Various bacteria species isolated from the gastrointestinal tracts of household rats in Abraka community

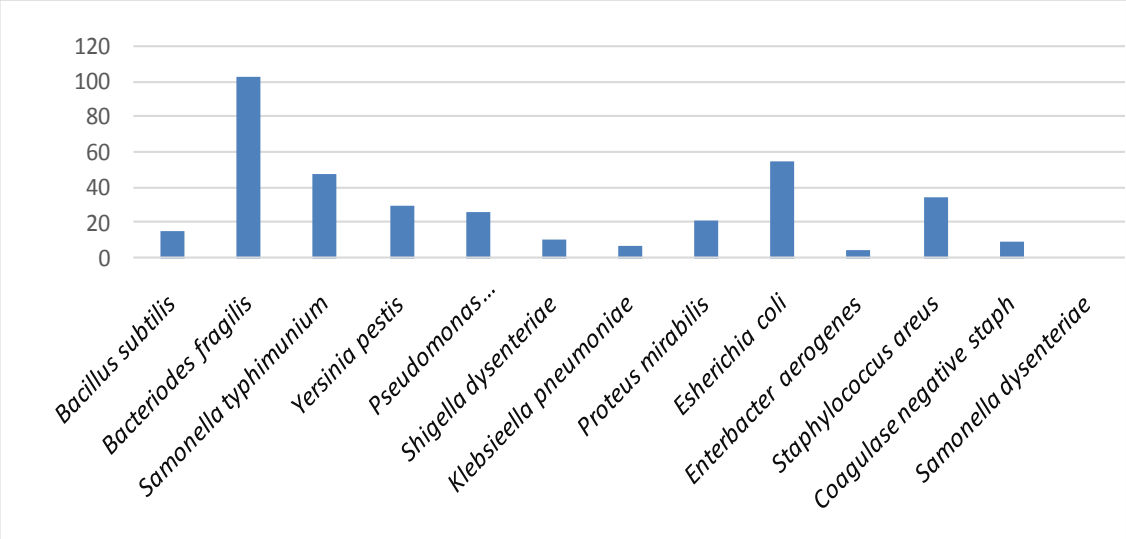
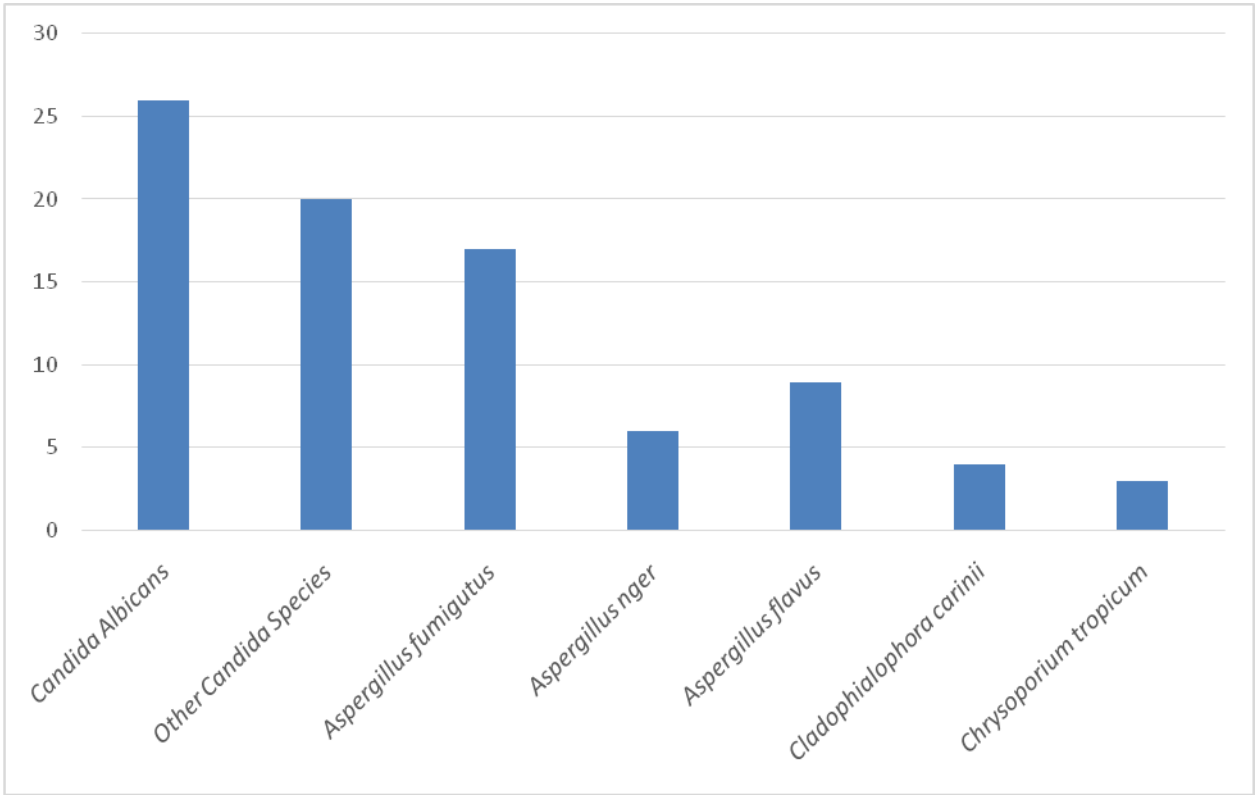


Fig.2 Various types of fungi isolates from the gastrointestinal tracts of household rats in Abraka community



Possible mechanisms for antibiotic resistance includes; (1) the presence of antimicrobial resistance genes (intrinsic resistance), (2) the selective pressure exerted by excessive antibiotic use by humans[28,29].

Our study implies that over 66%of bacteria isolates were resistant to commonly used antibiotic. This results is consistence with reports by Alermale Admas *et al.*, (2020), Melkanu Abebe Abebe *et al.*, (2019), Richard J. Fair and Yithak Tor (2014).[30],[31],[32] In contrast to our study, a lower percentage of 44.4% of total isolated organisms being resistant to commonly used antibiotics has been reported.[33]Speculated reasons for differences in antibiotic resistance pattern could due to techniques, equipment used for antibiotics resistant testing, rational use of antibiotics, and local resistant pattern.[34]

Of the 38 isolates that were tested against Vancomycin, an average of 3.8% were resistant. Rodriques, *et al.*, (2002) reported a lower average vancomycin resistance of 0.0% while higher percentages of 15 to 30% have been reported by other several other studies. [34,31,35,36,37].

There are little or scanty studies on the burden of organisms in the gut of house rats and its impact on house hold dwellers in our environment[38]. This study therefore serve as baseline study and may be useful for Health Practitioners, environmental impact assessment officers and policy makers. Household rats have long been a concern in rural areas, eating and contaminating stored food, spreading disease, and degrading the physical environment[39,11,27.]

The underreporting of rats zoonoses and, in many cases, inadequate attention paid to the diagnosis and management of these essential diseases are two main messages to emerge from this study[3,5].

When exploring ways to minimize the impact of rodents on rural livelihoods, there are two key issues to consider: 1) recognizing the current impact of rodents on food protection, health, and nutrition of communities, as well as existing awareness, behaviour, and practices of people regarding their rodent problems, and 2) designing cost-effective strategies that can be sustained.[38]

The results of this study should be interpreted with caution as it was done in a small community in Abraka, Delta State, Nigeria.

This therefore limits its generalization. Our techniques of disk diffusion as methods of resistance testing could also be prone to technical and observer's error.

Antifungi resistance testing testing were not included in our study due paucity of equipment. More extensive studies on burden of household rat borne pathogens using modern techniques are required in our environment.

Taken together, the result of this study indicate a high burden of bacteria, fungi and bacteria resistance organisms in household rats that can be transmitted to man.

It also suggest that increased reporting of this zoonotic disease coupled with proper diagnosis and management are ways of mitigating this burden.

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