# Enumeration of the Aerobic Bacterial Flora of the House Mouse (*Mus musculus*) in Akoko South West Local Government Area of Ondo State, Nigeria

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**Abstract**

*The gastro-intestinal tract of mammals is colonized by a diverse range of microorganisms. House mice (Mus musculus) commonly invade houses where they feed and defecate on foods (raw and processed). This study therefore investigated the gut microbiota of the mice to establish the likelihood of bacterial contamination of these foods by house mice. One hundred and fifty house mice comprising of 76 males and 74 females were trapped with mice-trap from four locations in Akoko South West Local Government area of Ondo State namely Akungba, Ayepe, Iwaro and AAU hostels. The microflora of two different parts (caecum and colon) of the mice's Gasstro Intestinal Tract were investigated using viable count and cultural methods while the isolates were identified using API kit. Disc diffusion method was used for the susceptibility assessment of the isolates. Mice samples from Ayepe and Adekunle Ajasin University hostels gave the highest microbial count of 27.0 x$10^3$ and 25.3x$10^3$cfu/$mm^2$ respectively. All the caecum samples gave higher count than their colon counterpart. Eight bacteria species namely Escherichia coli (125), Klebsiella sp (115), Salmonella sp (12), Shigella flexneri (10), Coagulase –ve Staphyloccoci (110), Citrobacter sp (37), Pseudomonas aeruginosa (30) and Lactobacillus sp (65) were isolated with E. coli having the highest frequency of isolation. The isolates (54%) were sensitive to the 4- Quinolones (Ciprofloxacin and Ofloxacin) while 25% were resistant to the Cephalosporins (Cefuroxime and ceftazidime). Poor activities were equally exhibited by Augmentin and Ampicillin. The microorganisms isolated from the GIT of the studied mice were known pathogens. The accesses of the house mice to food or drink items should be prevented through barrier erection and the use of rodent-proof bags for storage of foods and drinks. Prompt treatment is also recommended for infected individuals especially resulting from food contamination/ poisoning*.

**Keywords:** Mus musculus, microbiota, susceptibility test, GIT, Ondo State.

## INTRODUCTION

The house mouse (*Mus musculus)* is a tiny, hairy mammal with buldging eyes, small round ears, long tail and pointed nose. It is found in every habitable continent of the world. *Mus musculus* with other rodent species originated from Asia, from where they spread over the world along with the development of agriculture, which provided shelter and supplies of food (Hotchkiss and Vandenbergh, 2005) House mice are generally poor competitors and in most areas cannot survive away from human settlements in areas where other small mammals are present (Mead and Griffin, 1998). Mice are underground dwellers, omnivorous and can breed year-round when conditions are optimal. They manage well without water for a long substantial time and adapt to temperatures as low as $10^{o}$C (Hanney, 1975). As part of mice survival strategy, the female partner preferences usually adopted by them often enhance offspring ability to survive infectious diseases (Shirley *et al*., 2014).

Mice affinity for human settlements raises question about whether mice could be potent zoonotic agents. One way mice can cause the spread of various diseases includes the destruction and contamination of food items (Annette and Cleas, 2012; Masami *et al*., 1976). The consumption of food and water contaminated with the feaces of infected mice has been reported as a major source of human infection (Mead and Griffin, 1998). Apart from bacteria, an individual may be exposed to viral infections (Hantavirus disease, Mouse adenovirus, Hepatitis viruses, Parvovirus, Cytomegalovirus) by breathing contaminated dust after disturbing or cleaning rodent droppings or nests, or by living or working in rodent-infested settings. It could also be by touching something contaminated with rodent urine, droppings or saliva or eating foods contaminated with these products. Farm animals too are not spared of possible infection from infected mice since it is often difficult to exclude mice from animal houses.

Though, there are few studies on the subject of rodents as a risk of transmission of *Campylobacter* sp, one study concluded that occurrence of rodents was one of the risk factors for high *Campylobacter* prevalence in broiler chicken (Berndton *et al*.,1996) Other commonly reported zoonotic diseases associated with mice are the food borne enteric diseases of Salmonellosis and Yersiniosis (Annete and Eckner, 2010; Griffith *et al*., 2006). In the Netherlands, mice on pig farms were found to be carriers of Methicillin-Resistant *Staphylococcus aureus* (MRSA) of a multilocus sequence type 38 strain that has emerged as course of hospital acquired infection (van de Giessen, 2009). The two predominant causes of visitation to major health facilities in Akoko South West Local Government area of Ondo State are typhoid fever and malaria fever, the former being caused by *Salmonella enteric* serotype Typhi, one of the common bacteria isolated from the GIT of mice (Tatavarty *et al.*, 2012). Sources of water supply to the various communities in the studied area has been examined, a prevalence of 1.3% was reported for *Salmonella typhi*, a causative agent for typhoid fever (Olajubu and Ogunnika, 2014). Cizek *et al.*, (2000) reported that when infected with *Escherichia coli* 0157, the house mice can continue to shed the organism in their feaces for the next ten days and the shed organisms can survive for 34weeks. This study was therefore designed to assess the possible effect of mice feaces' contamination of foods (very common in these communities), infection by *Salmonella* sp and other pathogenic bacteria.

## MATERIALS AND METHODS

### Sampling

One hundred and fifty house mice comprising of 76 males and 72 females were trapped using mouse traps in four locations within Akoko South West Local Government area of Ondo State. The locations are Ayepe, AAU hostels, Iwaro and Akungba-Akoko.

### Sample processing

The mice were aseptically dissected on a disinfected dissecting table. A $10mm^2$ area of both the colon and caecum were swabbed separately and placed in 10mls of peptone water. The mixture was vigorously agitated and ten-fold serial dilutions were made out of which 1ml of each dilution was used to seed each of Plate Count agar, Salmonella-Shigella agar, Eosin Methylene Blue agar, Blood agar and McConkey agar in duplicate. The plates were incubated aerobically at $37^oC$ for 24hours. The colony count and identification of isolates were done at the expiration of incubation period. Colony counter was used to enumerate the colonies while the identification of isolates was done using API kit. The susceptibility testing of the isolates was done on Meuller-Hinton agar using the disc diffusion method.

## RESULTS

The spread of one hundred and fifty mice caught in five locations of Akoko South West Local Government area of Ondo State is presented in Table 1. Though the distribution is almost even, Akungba has the highest number of sample (36%).

Table 1: Sample Sites and Sex Distribution of Mice

| Sample Location | Sex Distribution | | Sub-Total |
|---|---|---|---|
| Akungba | Male | 20 | 44 |
| | Female | 24 | |
| AAUA Hostels | Male | 20 | 46 |
| | Female | 26 | |
| Ayepe | Male | 12 | 30 |
| | Female | 18 | |
| Iwaro | Male | 24 | 30 |
| | Female | 06 | |
| Total | | 100% | 150 |

The average colony count of the colonizing bacteria are shown in Table 2 with Ayepe and AAU hostels recording 27.0 $x10^3$ and 25.3 $x10^3$ $cfu/mm^2$ of sampled area. Samples from Akungba gave the lowest count of $14x10^3 cfu/mm^2$. The caecum samples gave a relatively higher count than the colon samples.

Table 2: Average Colony Count (cfu/$mm^2$) of Bacteria Isolates

| Sample Location | Colon | Caecum | Diff. |
|---|---|---|---|
| Akungba | $14x10^3$ | $14.5\ x10^3$ | 0.5 |
| AAUA Hostels | $18x10^3$ | $25.3x10^3$ | 7.3 |
| Ayepe | $20x10^3$ | $27x10^3$ | 7.0 |
| Iwaro | $17x10^3$ | $17x10^3$ | 2.0 |

Eight different aerobic bacteria were isolated from the sampled mice. The most frequently isolated bacteria were Escherichia coli (24.8%), *Klebsiella* sp (22.8%) and Coagulase –ve Staphyloccocus (21.8%) while *Salmonella* sp (2.4%) and *Shigella* sp (2.0%) were the least isolated. Other bacterial isolates are *Citrobacter* sp, *Pseudomonas aeruginosa* and *Lactobacillus* sp as shown in Table 3.

Table 3: Frequency of Bacteria Isolation

| Microorganism | Frequency | %Frequency |
|---|---|---|
| *Escherichia coli* | 125 | 24.8 |
| Coagulase –ve Staphylococcus | 110 | 21.8 |
| *Klebsiella* sp | 115 | 22.8 |
| *Salmonella* sp | 12 | 2.4 |
| *Shigella* sp. | 10 | 2.0 |
| *Citrobacter* sp. | 37 | 7.3 |
| *Pseudomonas aeruginosa* | 30 | 6.0 |
| *Lactobacillius* sp. | 65 | 12.9 |
| n = 504 | 504 | 100% |

Ciprofloxacin, Ofloxacin and Genticin showed good activities against most of the isolates while Ampicillin recorded the weakest activityas shown in Table 4.

Table 4: Antibiotic Susceptibility Test of the Isolates

| | Antibiotics | | | | | | |
|---|---|---|---|---|---|---|---|
| Organisms | CIP S (R) | CIP S (R) | AUG S (R) | CAZ S (R) | CRX S (R) | GEN S (R) | AMP S (R) |
| *E. coli* (125) | 105(20) | 115(10) | 110(15) | 120(5) | 95(30) | 115(10) | 15(110) |
| Coagulase –ve *Staphylococcus*(110) | 98(12) | 98(12) | 75(35) | 75(35) | 65(45) | 101(9) | 10(100) |
| *Klebsiella* sp.(115) | 25(90) | 31(84) | 10(105) | 10(105) | 10(105) | 25(90) | 2(113) |
| *Salmonella* sp.(12) | 12(0) | 12(0) | 8(4) | 6(6) | 6(6) | 10(2) | 0(12) |
| *Shigella* sp.(10) | 7(3) | 7(3) | 6(4) | 4(6) | 4(6) | 8(2) | 1(9) |
| *Citrobacter* sp(37) | 20(17) | 20(17) | 18(19) | 17(20) | 17(20) | 25(12) | 15(22) |
| *Pseudomonas* sp (30) | 17(13) | 17(13) | 10(20) | 28(02) | 3(27) | 5(25) | 3(27) |
| *Lactobacillus* sp (65) | 61(4) | 59(6) | 25(40) | 30(35) | 45(20) | 61(4) | 5(60) |

Key: CIP- Ciprofloxacillin (5ug); OFX- Ofloxacin (5ug); AUG- Augmentin (10ug); CAZ- Ceftazidime (10ug); CRX- Cefuroxime (10ug); GEN- Gentamycin (10ug); AMP- Ampicillin (10ug)

## DISCUSSION

The settlements in Akoko South West Local Government is that of a rural setting with old buildings and architectural designs that readily allow free movement of mice. One hundred and fifty mice samples were examined for their Gastro Intestinal Tract (GIT) colonizing bacteria. The male to female ratio (1:1) distribution of the 150 mice samples gave a good and equal assessment of the gender and the colonizing aerobic organisms. The caecum and colon samples from Ayepe gave the highest bacteria count from the study. This was expected being the remotest of all the sampled communities, with patches of bush around dwelling structures. Akungba was adjudged the neatest, but the most populated being a University town, hence the lowest count recorded was in order as mice like dirty environment which would likely increase the level of acquired microbes. Higher bacterial count from colon than caecum was reported by Masami *et al*., (1974), this is at variance with the present study which was done on wild mice as against his study on laboratory mice. The isolation of *Salmonella* sp from some of the samples is in agreement with some earlier studies of Mierburg *et al*., (2006) and Pocock *et al*., (2001). Similarly, there are documentations about the isolation of *E. coli* (Witold and Carolyn, 2011; Pritchett-Corning *et al*; 2009) *Staphylococcus aureus* (Annete and Eckner 2010; van de Giessen *et al*., 2009) *Klebsiella* sp. *Shigella* sp and *Citrobacter* sp (Annette and Cleas, 2012; Akande, 2008). The isolation of *Yersinia enterocolitica* from mice GIT has been reported widely (Backhans *et al*., 2011; Jalava *et al*., 2006) though not isolated from this study. Information about Yersinia infection in the studied community has not been documented, this might be responsible for the non-isolation of this widely reported bacterium from mice. The isolation of these organisms becomes

important in view of their documented roles in disease initiation. The isolation of *E. coli* becomes important because some strains of *E. coli* have the ability to cause human diseases, colonizing animal carriers and surviving in the environment (Vanaja *et al*., 2009; Roldgaard *et al*., 2004). They produce extremely potent cytotoxins that enter host cells expressing toxin receptor and blocking protein synthesis by irreversibly damaging ribosomal RNA (Endo *et al.*,2008) A review by Witold and Carolyn (2011) indicated that *E. coli* of the strain EHEC 0157:H7 are equally found in domesticated ruminant animals. There was however, no significant difference in the distribution of microorganisms among male and female mice. Contamination of foods and drinks by the droppings of these 'carrier mice' might contribute to the reported cases of typhoid fever in hospitals around the studied environment. A study of water sources (wells and boreholes) in this area have been reported to be variedly contaminated with microbes including *Salmonella* sp (Olajubu and Ogunnika, 2014) Antimicrobial resistance to multiple drugs is a growing problem in many community bacterial isolates. In this study, except for Ampicillin, greater percentages of *E. coli* tested were sensitive to most of the antibiotics with the best activity recorded in ceftazidime. The activity of the 4-quinolones against Salmonella isolates was total and no activity by Ampicillin. Ampicillin has long been abused and the production of sub-standard brands because of high demand might have affected its in vitro performance too. Ciprofloxacin and Ofloxacin (4-quinolones) though common in pharmaceutical shops still give some measure of hope as demonstrated by their in vitro activity against most of the isolates. Gentamycin gave good activity against all the bacterial isolates except *Klebsiella* sp on which it performed poorly. Further studies have been initiated on this unusual observation. Gentamycin is not often abused because of its presentation in injectables; this might contribute to its in vitro efficacy.

## CONCLUSION

The isolation of some pathogenic bacteria (*Salmonella* sp, *E. coli*, *Shigella* sp) from the colon and caecum of house mice reinstate their contaminating zoonotic role in the spread of some diseases like typhoid fever. This calls for erection of barriers to disallow these home-friendly animals, free access to homes. Rodent-proof bags should be used for storage of food and drinks while prompt and adequate treatment is recommended for infection

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