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Isolation and Antimicrobial Susceptibility Pattern of *Escherichia coli* from Rectal Swabs of Rodents trapped from household compounds in Wolaita Zone, Southern Ethiopia

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Abstract: Rodents are also considered as reservoir hosts for wide range of pathogens of zoonotic importance. *Escherichia coli* has been isolated from rodents in different parts of the world. To isolate and test antimicrobial susceptibility pattern of *Escherichia coli* from synanthropic rodent species trapped from household compounds by using rectal swab. A total of 77 rodents were trapped and standard methods were used to isolate *E.coli* from all the rodent species comprising *Stenocephalemys albipes* 24(31.2%), *Mus mohamet* 18(23.4%), *Arvicanthis spp* 15(19.5%), *Mastomys erythroleucus* 12(15%), *Gerbriliscus species* 4(5.2%), *Crocidura oliveri* 3 (3%) and *Acomys wilsoni* 1(1.3%) were analyzed for antimicrobial susceptibility to twelve commonly used antimicrobials. All the 77 (100%) rectal swabs were positive for *E. coli*. The antibiogram revealed that 31.38% of the *E. coli* isolates were resistant to all drugs tested except Ciprofloxacin, Gentamicin and Chloramphenicol. Complete resistance to amoxicillin and Amoxicillin-clavunic acid was observed in the *E.coli* isolates. The results of this study demonstrated that rodents in the household compounds may have been exposed to materials containing antimicrobial residues and that rodents carry antimicrobial resistant bacterial organisms which can pose a public health hazard.

Keywords: *Escherichia coli*, antibiotic susceptibility pattern, synanthropic rodents, Wolaita Zone.

1. INTRODUCTION

Rodents are considered to be serious crop pests and damage crops of all types at both pre and post-harvest settings globally ^[1]. Rodents are also considered as reservoir hosts for wide range of pathogens of zoonotic importance. Human induced landscape changes contribute for emergence of rodent borne microparasites such as viruses and bacteria ^[2]. Agriculture based habitat alteration such as deforestation and dividing land for household members are reported to favour presence of synanthropic rodent species and the pathogens they harbor such as *Bartonella* and hanta viruses in developing countries ^[3]. For instance, rodent-borne *Yersinia pestis* causes outbreaks of plague in Africa ^[4,3]. *Bartonella* and RNA viruses have been isolated from synanthropic rodents in Northern Ethiopia ^[1].

Escherichia coli has been isolated from rodents in different parts of the world ^[4]. The transmission dynamics of *E. coli* among synanthropic rodents, livestock and humans may contribute for the development of antimicrobial resistance ^[5-7].

Despite there are conducive conditions for rodent occurrence such as poor hygiene and traditional crop storage in rural household compounds in Ethiopia, there is no published data on isolation and antimicrobial susceptibility pattern of *E. coli* from synanthropic rodents. Considering the potential public health risk posed by rodents to livestock, pet animals, and humans, the current study was conducted to isolate and test antimicrobial susceptibility pattern of *E. coli* from rectal swabs of rodents trapped from rural household compounds in Fate and Abaya Chokare kebelles of Damot Gale and Humbo districts, respectively in Wolaita Zone, Southern Ethiopia.

2. MATERIALS AND METHODS

2.1. Study area

A total of 2 rural kebeles that are located in Humbo (geographical coordinates: 37N0382846, UTM 0729850; elevation: 1186 MSL) and Damot Gale (geographical coordinates: 37N0367959, UTM 0769846; elevation: 2073 MSL districts were selected purposefully for trapping of rodents based on consultation with district agricultural bureaus about the general hygiene profiles and rodent problems in the areas. A total of 10 household compounds (5 from Fate kebele and 5 from Abaya Chokare) were selected for trapping the rodents for screening of *Escherichia coli*.

2.2. Rodent collection

Rodents were trapped live by using Sherman LFA live traps (7.5x9.0x23.0 cm, HB Sherman trap, Tallahassee, USA) baited with peanut butter from 5 purposively selected household compounds from each kebele. Trapped animals were then transported to the Biomedical Science laboratory of the Department of Biology, Wolaita Sodo University. The animals were sacrificed by cervical dislocation and external body measurements (weight, lengths of the body, ear, tail and hind foot were recorded). Sex and species of the trapped animals were identified with expert guidance.

2.3. Collection of rectal swabs

Sterile saline-moistened cotton-tipped applicator sticks were used to collect rectal swabs from each of the rodent. The rectal swab from each rodent was transferred into sterile buffered peptone water in sterile screw capped test tubes and stored in 4°C refrigerator until culturing the target bacterium, *Escherichia coli*.

2.4. Isolation of *Escherichia coli*

The rectal swab from each rodent was inoculated onto MacConkey agar (Oxoid Ltd., Detroit, Michigan, USA) and Xylose-Lysine Deoxycholate Agar (Oxoid Ltd., Detroit, Michigan, USA) and incubated aerobically for 24 h at 37°C.

2.5. Antimicrobial susceptibility test

Modified Kirby-Bauer disk diffusion method was

used to test the susceptibility of *Escherichia coli* isolates to 12 different antimicrobial agents including Ampicillin (Amp, 10 µg), Tetracycline (Tet, 30 µg), Chloramphenicol (Chl, 30 µg), Nitrofurantoin (F, 100 µg), Cloxacillin (Ob, 5 µg), Amoxicillin (Amx, 2 µg), Nalidixic acid (Na, 30 µg), Gentamycin (Gen, 30 µg), Ceftazidime (Caz, 30 µg), Ceftriaxone (Cro, 30 µg), Ciprofloxacin (Cip, 5 µg), and Amoxicillin-clavulanic acid (Amc, 30 µg) (Oxoid, UK). The inocula were prepared by growing *E. coli* on MacConkey agar plates and 3-5 colonies from the plate were transferred with inoculating loop into 5 ml of Trypticase soya broth (BBL™ Trypticase™ Soya Broth, BIOTECH) and incubated at 35°C for 4-6 hours. The density of the suspension was adjusted by adding the bacterial suspension to a sterile saline tube to match the density of the desired 0.5 McFarland standards. The surface of Muller-Hinton agar plate was evenly inoculated with the organisms using a sterile swab. The diameter of zone of inhibition was measured by using

digital caliper and classified as sensitive (S), intermediate (I), or resistance (R) according to the standard recommendations of the Clinical and Laboratory Standards Institute [8].

3. RESULTS

3.2. Abundance of synanthropic rodent species and isolation rate of *Escherichia coli*

Out of 77 small mammals trapped for screening of *Escherichia coli*, 74 were rodents belonging to the species *Stenocephalemys albipes* (31%), *Mus Mahomet* (23%) and *Arvicanthis* (19%), *Mastomys erythroleucus* (15%), *Gerbriliscus* species (5%), *Crocidura oliveri* with (3%) and *Acomys wilsoni* (1%). All the 77 rodents examined were found to be positive for *E. coli*. The percentage of *E. coli* isolates was directly proportional to the number of each rodent species. *Stenocephalemys albipes* (n=24; 31.2%), *Mus Mahomet* (n=18; 23.4%) and *Arvicanthis* (n=15; 19.5%) was the most frequently trapped rodent followed by (Figure 1).

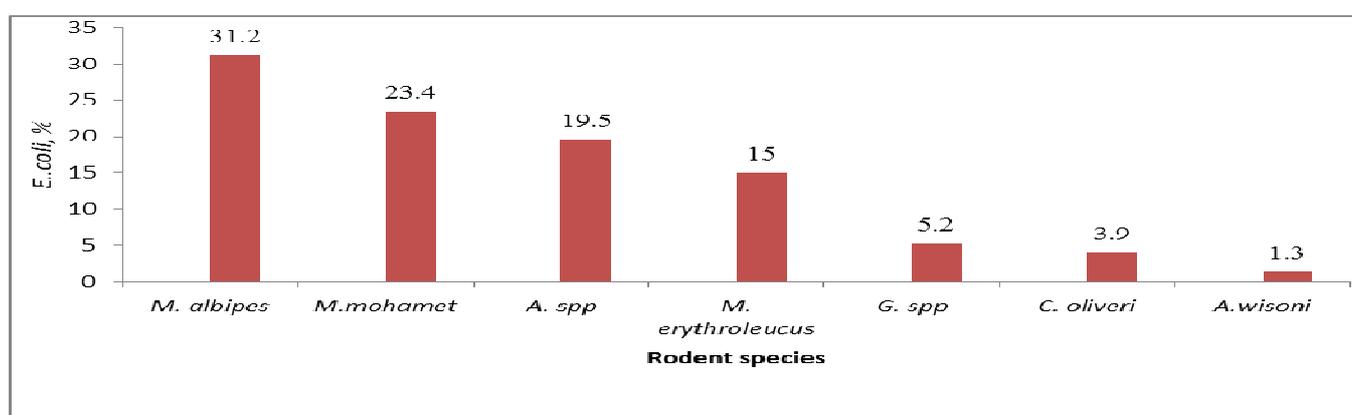


Figure 1: Abundance of rodent species and isolation rate of *E. coli*

3.3. Antimicrobial susceptibility pattern of *Escherichia coli* isolates from synanthropic rodents

The *Escherichia coli* isolate from *Arvicanthis* was found to be resistant for 7 antimicrobial agents out of the 12 antimicrobial drugs used for susceptibility test. Moreover, *E.*

coli isolates from *Crocidura oliveri* and *Mastomys erythroleucus* were found to be resistant for 6 antimicrobial drugs among the 12 antimicrobial drugs used for susceptibility test.

Table 1: Summary of antimicrobial susceptibility pattern of *E. coli* isolates from rodents collected from households in Wolaita Zone from December 2015 to March 2016.

Total No. of isolates tested	%	R	%	I	%	S	Conc.	Antimicrobial Discs
77	100	77	0.00	0	0.00	0	30µg	Amx
77	0.00	0	0.00	0	100	77	5 µg	Cip
77	0.00	0	2.59	2	97.40	75	30µg	Gen
77	2.59	2	3.89	3	93.5	72	30µg	Na
77	3.89	3	11.7	9	84.4	65	100µg	F
77	19.48	15	7.79	6	72.72	56	30µg	Tet
77	5.19	4	0.00	0	94.80	73	30µg	Caz
77	98.70	76	0.00	0	1.29	1	5 µg	Ob
77	5.19	4	0.00	0	94.80	73	30µg	Cro
77	46.7	36	45.45	35	7.8	6	10 µg	Amp
77	100	77	0.00	0	0.00	0	30µg	Amc
77	0.00	0	0.00	0	100	77	30µg	Chl
924	31.82	294	5.95	55	62.23	575		Total

Key: Conc = Concentration; Amx = Amoxicillin; Cip = Ciprofloxacin; Na = Nalidixic acid; Amp = Ampicillin; Gen = Gentamicin; F = Nitrofurantoin; Caz = Ceftazidime; Ob = Cloxacillin; Cro = Ceftriaxone; Tet = Tetracycline; Amc = Amoxicillin-clavulanic acid and Chl = Chloramphenicol.

The rest of the isolates from different rodent species had lower resistant rates ranging from 3-5 antimicrobial agents. All *E. coli* isolates from different rodent species had shown 100% resistance to amoxicillin and amoxicillin-clavulanic acid. On the other hand, all *E. coli* isolates from different rodent species had shown 100% resistance to amoxicillin and amoxicillin-clavulanic acid (Table 1).

4. DISCUSSION

In developing countries like Ethiopia, the sanitary profile of rural household compounds is poor and shared by human and domestic animals. In addition, there are synanthropic rodents that inhabit rural household compounds. These rodents include those that naturally live inside houses such as the black house rat, *Rattus rattus*, and those that migrate to the human settlement areas as a result of fragmentation or loss of their habitats due to human induced land-use land-cover changes. Synanthropic rodents that share rural household compounds are considered to pose public health and veterinary risks [2]. The present study attempted to screen synanthropic rodents for *Escherichia coli* and testing antimicrobial susceptibility pattern of *E. coli* isolate for 12 commonly used antimicrobial agents in Ethiopia.

All the 77 rodents screened were positive for *Escherichia coli* with 100% isolation rate. This result was comparable with a similar study elsewhere in Nigeria [6] and Trinidad and Tobago [5]. However, the isolation rate of *E. coli* in this study was higher than earlier studies in Kenya [3]. The difference in isolation rates of *E. coli* from rectal swabs of rodents trapped in different countries may be attributed to the underlying difference in the population of *E. coli* in the gut of rodents and the sensitivity of culturing technique used to isolate the bacterium.

Among the 12 antimicrobial agents tested, *Escherichia coli* isolates from all of the rodents screened were shown complete resistance to amoxicillin and amoxicillin-clavulanic acid. It is important to note that amoxicillin and amoxicillin-clavulanic acid are used as "first" line agents for treatment of bacterial infections in humans. In similar study done in Nigeria were shown 57% of *E. coli* isolates of rodents to be resistant against antimicrobial agents including amoxicillin. In the present study, the finding that ciprofloxacin and chloramphenicol appeared to be the most potent antibiotics against *E. coli* (based on the fact that no isolates demonstrated resistance except two intermediate isolates to gentamicin) which is in consistency with previous reports in Nigeria [10]. Among the 77 rodents trapped for screening of *E. coli* belonging to order rodentia and family muridae. The rodents belong to species *Stenocephalemys albipes* 24(31.2%), *Mus mohamet* 18(23.4%), *Arvicanthis* 15(19.5%), *Mastomys erythroleucus* 12(15%), *Gerbriliscus species* 4(5.2%), *Crocidura oliveri* 3(3%) and *Acomys wilsoni* 1(1.3%). All of the synanthropic rodents trapped inhabit human modified land scapes including scrub lands, grasslands as well as agricultural lands.

The animals are also reported to migrate to the human settlement areas and occur as commensal species in close association to humans [12]. This can imply that the animals can potentially carry wide range of potentially zoonotic pathogens including *E. coli* to the human settlement

areas. Such rodents can interact with house rats, domestic animals and humans and can play substantial role in the epidemiology of infectious diseases of public health and veterinary importance [13]. Furthermore, such findings have implications for human and veterinary medicine regarding antimicrobial usage and subsequent selection of antimicrobial-resistant organisms. However, the possibility of being carriers of other uncultured pathogens, multiple drug resistant *E. coli* strains and molecular basis of resistance acquisition and transmission also cannot be ignored.

5. CONCLUSIONS AND RECOMMENDATIONS

This study demonstrated that rodents in a household compound may be exposed to materials containing antimicrobial residues and that rodents carry antimicrobial resistant bacterial organisms which can pose a public health hazard. Furthermore, such findings have implications for human and veterinary medicine regarding antimicrobial usage and subsequent selection of antimicrobial-resistant organisms. Therefore, the study highlights the need for implementation of integrated rodent control that pose public health risks in the study area.

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