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RESEARCH OPINIONS IN ANIMAL & VETERINARY SCIENCES

Frequency of detection of *salmonella spp.* and *Esherichia coli* in the faeces of house rat in Abia State, Nigeria

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Abstract

This study was carried out to determine the frequency of isolation of *Salmonella spp.* and *E. coli* 0157 in the faeces of rats obtained from three local government areas in Abia State Nigeria, as well as to evaluate their antimicrobial resistance. A total of 180 rates were trapped and standard methods were used to isolate *Salmonella* and *E. coli* 0157. The *E. coli* was characterized by use of blood agar to determine haemolytic and mucoid colonies and using *E. coli* 0157 antiserum to determine 0157 strain and use of sorbitol MacConkey agar to determine non sorbitol fermentation. Ten antimicrobial agents were used to determine resistance via disc diffusion method. Out of a total of 180 trapped rats, 16 (8.0%) were positive for *Salmonella* while 158 *E. coli* isolates tested positive. 0 (0.0%), 22 (14.5%) and 16 (10.1%) were haemolytic, mucoid and non-sorbitol fermenters respectively. All the isolates proved negative for 0157 strain. The frequency of resistance to the 10 antimicrobial agents tested was 30.1% (48) for *E. coli* and 75.7% (13) for *Salmonella spp.*

Keywords: *Samlonella spp., E. coli, house* rats, Abia State, Nigeria

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Introduction

Human salmonellosis is a major public health problem. Salmonellosis caused by *Salmonella enterica* serovar *Enteritidis* is the most prevalent cause of bacterial food poisoning in Japan. NIIL (2003), zoonesis which could be caused by bacterial pathogens have represented a burden to human health throughout times (Khan et al., 2008), and (Richt et al., 2009). Rats contaminate food and transmit diseases to other animals and humans (Meerburg et al., 2009). Their activities, therefore, have a public health and economic implications because of the zoonotic agent they transmit (Inoue et al., 2008) (4-7). Rodents are public health hazards since they can be reserviours for human salmonellosis (Hilton et al., 2002).

Esherichia coli (E. coli) have been reportedly isolated from several wildlife species including free roaming rodents in domestic and rural area (Adesiyun et al, 1994). A number of phenotypic and other characteristics of *E. coli* isolated from various household have been described. Some of these characteristics include mucoid and haemolytic properties (Prada et al., 1991). A majority of *E. coli* 0157; H_7 serotypes are also known to be non-sorbitol fermenters (March et al., 1986). Recently, *E. coli* 0157: H_7 has emerged as a major food-borne, zoonotic pathogen in humans, responsible for haemorrhagic colitis and haemolytic uraemic syndrome (Karmali et al., 2010). In Abia State Nigeria, a study was conducted to evaluate the frequency of selected pathogen including *E. coli* 0157 serotype and *Salmonella spp.* in free roaming rats in Aba city and to determine the frequency of resistance to antimicrobial agents. This study was necessitated due to the series of complaint by residence of the city of increasing rat population in their places of abode and to determine whether rats are reservoir source for transmission of *E. coli* and *salmonella species* to humans.

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Materials and Methods

Trapping of rats took place in several locations among which include notable restaurants, feed deports and residential areas following observable number of rats in the densely populated Aba city. All the rats caught during the day were transported to the laboratory in a closed plastic basket within 2 hour.

The material used for trapping of the rats is called magic gum. The magic gum is a product locally made and functions by trapping any rat that passes where it's positioned and rendering it incapacitated without necessary killing it. The hairs of the rat get firmly glued to the gum. The trap is made of thick paper with gum centrally placed.

Collection of samples from rats

In the laboratory, the rats were pulled out carefully from the gum using forcep and scapel. The use of 10% ketamine hydrochloride (Dutch farm, veterinary pharmaceutical CPY Holland) as anaesthesia and xylazin marketed as chalazin 2% solution was administered into the rats. The approximate dosage was 85mg. Ketaimine mixed with 15mg xylazine per kg of rats intramuscularly (White and Wixson 1987).

After a while, there was marked loss of sensation and reflex. The abdominal cavity was incised and reflected using surgical blade and a pair of forcep and the gastrointestinal tracts were removed and place in a disposable petri-dish.

Faecal samples culture

The gastrointestinal tract of each rat was cut open and all the content from the small intestine to the caeca of the rats were removed. Swabs of the intestinal content were then plated to MacConkey agar (MAC), (oxoid Ltd., Detroit, Michigan, USA) and eosin methylene blue (EMB) agar from the same company and incubated aerobically at 37^{o}C for 24 hours. This is for detection of *E. coli.* Sterile loopful of characteristics colonies on EMB agar (metallic green sheen) and pinkish colonies on MAC agar was subjected to biochemical tests for identification of *E. coli* using standard methods of Mifadden (2000).

The isolates of *E. coli* were inoculated and plated on blood agar and sorbitol MacConkey (SMAC) agar plates, and incubated overnight at 37^{o}C. Typical phenotypic characteristics of *E. coli,* specifically mucoid appearance and haemolysis on blood agar plates and the ability to ferment sorbitol on SMAC agar as described earlier (March et al., 1986) was observed with the use of *E. coli* 0157 antisera (Oxoid Ltd, Michigan, Ohio, USA). 0157 serotypes were detected among 4 to 6 *E. coli* isolates per agar plate using the slide agglutination test.

***Salmonella* isolation**

Approximately 1g of intestinal contents of rats was added to 9ml of selective enrichment broths (selenite F-broth) and mixed thoroughly by turning the test tube (shaking) and then incubated at 37^{o}C overnight. The broths were later sub cultured onto brilliant green agar (BGA) (oxoid Ltd, Detroit, Mich., USA) and incubated aerobically at 37^{o}C for 24 hours. On examination, suspected isolates (4 to 6) of *Salmonella spp.* showed pink colonies. They were subjected to biochemical tests using standard methods (Mcfadden, 2000). Biochemically identified *Salmonella* isolates were subjected to slide agglutation test using commercially available *Salmonella* polyvalent antiserum (Difio Ltd., Detroit, Mich., USA). All isolates that were positive by the slide test were recorded.

Antimicrobial resistance determination

The resistance of isolates of *Salmonella spp.* and *E. coli* to ten antimicrobial agents was evaluated using the disc diffusion method. The antimicrobial agents and the concentrations used were as follows: ampicillin (AMP, 10µg), cephalothin (Cth, 30µg), gentamycin (gen 10µ), tetracycline (Tet, 30µg), streptomycin (str 10µg), nalidixic acid (Na, 30µg), Kanamycin (Kan, 30µg), chloramphenicol (chl, 30µg), norfloxacin (Nf, 10µg) ciprofloxacin (cp, 5µg).

The recommended method by National Committee for Clinical Laboratory Standards guidelines (NCCLS, 2002) were used to determine resistance or susceptibility of isolates. The isolates that display resistance based on their zones sizes of inhibition were classified as resistance isolates.

Statistical Analysis

The frequency of isolation of the two bacteria tested as well as the prevalence of resistance to the ten antimicrobial agents tested were compared and then evaluated using chi-squared test (X^2) (Cole et al., 1989). The degree of significance was determined at 5% confidence interval.

Results

Frequency of isolation of *E. coli* and *Salmonella Spp.*

A total of 180 rats were trapped. The intestinal content was positive for *Salmonella* 16 (8.0%) while *E. coli* were positive to the tune of 158 (80.0%). The difference in the frequency of isolation was significant 05: X^2 as shown in table 1.

Characteristics of bacterial isolates

Table 2 shows the characteristic features of *E. coli* isolates among which the 158 isolates tested, 22 (14.5%) were mucoid and 16 (10.1%) were non

Fig. 1: Shows the geographical locations across Abia State where the study was carried out. (Rats were trapped). This is suggestive of convenience sampling.

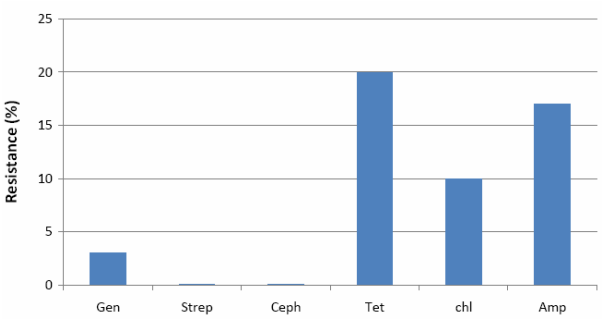

Fig. 2: *E.Coli* Isolation with Various degree of Resistance to Antibiotics.

Gen: Gentamycin; Strep: Streptomycin; Ceph: Cephalothin; Tet: Tretracyclin; Chl; Chloramphenicol; Amp: Ampicillin

Table 1: Frequency of isolation of *Salmonella spp.* and *E. coli* from the fecal sample of rats. Frequency of Isolation

Aba South LGA		*Salmonella spp.*	*E.coli*
Residential areas	A	2	18
	B	1	10
	C	3	20
Aba north LGA			
Restaurants	J	4	25
	K	1	21
	L	1	24
Osisioma LGA			
Feed deport.	X	1	18
	Y	3	12
	Z	0	10

Characteristics of bacterial isolates

Table 2: Characteristic a feature of E. coli isolates of the 158 isolates tested, 22 (14.5%) were mucoid and 16 (10.1%) were non sorbitol fermenters

Isolate tested	Total no (+)	% of isolates (+)	Mucoid
158	22	14.5	Mucoid
158	16	10.1	Non sorbitol fermenters
158	Nil	0.0	Haemolysis

Total *E. coli*; +=positive, -=negative, nil=zero; All *Salmonella* isolates were negative for haemolysis

Table 3: Number of isolates and percent resistance of E. coli and Salmonella spp.

	No of isolates	Total no of isolates	% of resistance
Prevalence of resistance for *E.coli*	48	158	30.1%
Prevalence of resistance *Salmonella spp.*	13	16	75.0%

sorbitol fermenters. However, all *Salmonella* isolates were negative for haemolysis.

Of the 158 isolates of *E. coli* and *Salmonella spp.* tested, 62 (39.1%) showed resistance to one or more of the antimicrobial agents tested (Table 3). The frequency of resistance was 30.1% (48 of 158), and 75.0%) (13 of 16) for *E. coli* and *Salmonella spp.* respectively. The difference was statistically significant (0.5: X^2). In *E. coli* isolates, the resistance to streptomycin (0.0%), cephalothin (0.0%) and gentamycin (3.0%) while (16.4%) ampicillin and chloramphenicol (10.0%). Of the 16 *Salmonella* isolates, three isolates were resistance to ampicillin, nalidixic acid, tetracycline and chloramphenicol, and they were all susceptible to the six remaining antimicrobial agents tested.

Discussion

The frequency of *Salmonella* and *E. coli* in the fecal matter of *R. rattus* was studied. Rats mostly live in house sewage or animal farms from where they can pick *E. coli* by entering contaminated feed, water and waste through poultry and cattle dung. In this study, *E. coli* was the most frequently isolated and identified from the fecal material of *R. rattus*. This is in agreement with Cizek et al. (1999) who reported that 5.3% of stool samples of rodents were positive for *E. coli* in Czech Republic. It was no surprise that *E. coli* strains were isolated from the gastrointestinal tracts of the rats studied as they constitute a major group of the family Enterobactericea in animals (Glyles, 1993).

The prevalence of 80.0% found in rats in the current study is slightly higher than 51.2% reported for rodents at the local zoo in Trinidad (Gopee et al., 2000). It is well known that animals harboring *Salmonella* contaminate their environment, which can become the

source of infection for both human and animals Humpgery (2001).

The frequency of resistance, in relation to antimicrobial agents was 20.0% for tetracycline, 16.4% for ampicillin and 10.0% for chloramphenicol. This was lower than the rate reported in Trinidad. The observed low frequency of resistance to gentamycin (3.0%), cephalothin (0.0%), and streptomycin (0.0%) is in agreement with published reports on mammalian wildlife in Trinidad and Tobago (Adesiyun et al., 1999).

The 16 isolates of *Salmonella spp.*. as reported in this study exhibited resistance to ampicillin, tetracycline, chloramphenicol and nalidixic acid. Despite the fact that the *Salmonella* isolate was low, it has been implicated that rodents serve as source of muti-resistant *Salmonella spp.* This resistance to antimicrobial agents have been reported Swanson et al. (2007).

Conclusions

Summarily, the rats were seen to be carriers of microorganism at a relatively low frequency for *Salmonella* and fairly high frequency for *E. coli*. Since rats live and have close contact with human and pet animals, they may present health risk to human, pet animal and domestic livestock hence the need to take proactive measures to eradicate them is very necessary.

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