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*Research Article*

# Frequency of Detection of *Escherichia coli*, *Salmonella* spp., and *Campylobacter* spp. in the Faeces of Wild Rats (*Rattus* spp.) in Trinidad and Tobago

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The study was conducted to determine the frequency of isolation of *Salmonella*, *Campylobacter* and *E. coli* O157 in the faecal samples of rats trapped across the regional corporations in Trinidad and to assess their resistance to antimicrobial agents. A total of 204 rats were trapped for the detection of selected bacteria. Standard methods were used to isolate *Salmonella*, *Campylobacter* and *E. coli* O157. Characterization of *E. coli* was done on sorbitol MacConkey agar to determine non-sorbitol fermentation, blood agar to determine haemolytic and mucoid colonies and by using *E. coli* O157 antiserum to determine O157 strain. The disc diffusion method was used to determine resistance to nine antimicrobial agents. Of the 204 rats, 4 (2.0%), 7 (3.4%) and 171 (83.8%) were positive for *Salmonella* spp., *Campylobacter* spp. and *E. coli*, respectively. Of the 171 isolates of *E. coli* tested 0 (0.0%), 25 (14.6%) and 19 (11.1%) were haemolytic, mucoid and non-sorbitol fermenters, respectively. All isolates were negative for the O157 strain. The frequency of resistance to the 9 antimicrobial agents tested was 75% (3 of 4) for *Salmonella*, 85.7% (6 of 7) of *Campylobacter* spp. and 36.3% (62 of 171) for *E. coli* ($P < .05$; $\chi^2$).

## 1. Introduction

Zoonoses which could be caused by bacterial pathogens have represented a burden to human health throughout times [1, 2]. Rats (*Rattus* spp.) contaminate food and transmit diseases to other animals and humans [3]. Their activities therefore pose both economic and public health implications, particularly with the zoonotic agents they transmit [4–7].

*Escherichia coli* (*E. coli)* has been reportedly isolated from several wildlife species including free-roaming rodents in domestic and rural areas, bats, farmed and wildlife in zoological gardens [8–10]. A number of phenotypic and other characteristics of *E. coli* isolated from various wildlife have been described. Some of these characteristics include mucoid and haemolytic properties which have been suggested to be virulence markers [11, 12]. A majority of *E. coli* O157:H7 serotypes are also known to be nonsorbitol fermenters [13]. In recent years, *E. coli* O157:H7 has emerged as a major food-borne, zoonotic pathogen in humans, responsible for the haemorrhagic colitis and haemolytic uraemic syndrome [14].

Rodents have been reported to be reservoirs of different serotypes of *Salmonella* spp. and have been implicated in contaminating foods with the pathogen and transmitting the pathogen in livestock farms [15, 16]. Rodent-borne salmonellosis has also been reported in humans [17, 18]. Failure to control rodent populations in some geographical locations has continued to pose health problems to humans with particular reference to salmonellosis and other pathogens [19, 20].

*Campylobacter* spp. have been isolated from various animal species, but avian species, particularly poultry are important reservoirs of *Campylobacter* spp. [21, 22]. Meerburg et al. [23] reported on the isolation of *Campylobacter* spp. from house rats and wild brown rats in the Netherlands

on organic farms. Wild rats therefore could act as reservoir or sources of *Campylobacter* spp. for livestock and humans.

Resistance of pathogens associated with wildlife including rodents has been documented, and it has been suggested that they may acquire or spread resistant strains to humans and livestock [24]. The chemotherapeutic implication for humans, livestock and pet animals can therefore not be ignored.

In Trinidad and Tobago and the Caribbean region, pathogens including *E. coli*, *Salmonella, Campylobacter* spp., leptospires, and hantavirus have been documented in rodents and other wildlife [25–28].

Considering the potential public health risk posed by rodents to livestock, pet animals, and humans because of their presence in rural and urban populations and closeness to humans, the current study was, therefore, conducted to determine the prevalence of selected pathogens (*E. coli* including the O157 serotype, *Salmonella* spp., and *Campylobacter* spp.) in free-roaming rats in Trinidad and to determine the frequency of resistance to antimicrobial agents amongst the isolates of the pathogens.

## 2. Materials and Methods

*2.1. Sample Size Determination.* The sample size of rats to be sampled was determined using the prevalence of 10% for *Salmonella* spp. infection for urban rats (*Rattus norvegicus*) as described by Hilton et al. [29] and a precision rate of 4%. The following formula [30] was used: $\text{No} = t^2(p)(1-p)/d^2$, where $t = 1.96$, $d$ = desired level of precision, 004, and $p$ = prevalence. An estimated sample size of 216 was determined.

*2.2. Source of Wild Rats.* The investigation was conducted between January 2006 and August 2006 when rats were randomly trapped at various locations across Trinidad as shown in Figure 1.

*2.3. Trapping of Rats.* The study was part of a larger study designed to determine the serovars of *Leptospira* serologically and by culture in rats [28]. The rodent control units of each of the Regional Heath Authority covering 7 counties in the country assisted in trapping rats using metal live catch traps with baits of cheese, fish, and other food items. The trappings took place in rat-infested areas such as surroundings of fast food restaurants and other eating establishments, food markets, and residential areas following complaints by members of the population. All rats caught during the day were transported to the laboratory, covered with ventilated bags to reduce the excitement of trapped rats, and transported to the laboratory within approximately 2 h after which the animals were trapped. Rats caught overnight were transported to the laboratory soonest possible in the morning. The age (adult or juveniles) and sex of each caught rat were noted as well as the geographical location of which it was trapped.

*2.4. Collection of Samples from Rats.* In the laboratory, the caged rats were covered with a black bag and rendered unconscious by the introduction of carbon dioxide from a pressurized tank into the sealed bag. Unconsciousness was determined by the evidence of lateral recumbency and the loss of pedal reflex. This was immediately followed by anaesthesia which was achieved by the use of a combination of a 10% ketamine solution (Dutch Farm Veterinary Pharmaceutical Company, Loosdrecht, Holland) and xylazine marketed as Bromazine 2% solution (Bomac Laboratories, Wiri Station Road, Manukau City, Auckland, New Zealand). For most rats, approximately the minimal dosage administered intramuscularly was 85 mg ketamine mixed with 15 mg xylazine per kg of rat [31], but more of the solution was administered, to affect rats until no response to pain and the loss of reflex were observed. The abdominal cavity was exposed using a surgical blade and a pair of forceps and the gastrointestinal tracts were removed and put in sterile Plastic Petri dishes as recommended by the Guidelines of the Canadian Council of Animal Care.

*2.5. Bacteriological Culture of Faecal Samples.* The gastrointestinal tract was cut open to remove all the content from the small intestine to the caeca of the rats. For the detection of *E. coli*, swabs of the intestinal contents were plated onto MacConkey agar (MAC), (Oxoid Ltd., Detroit, Michigan, USA) and eosin methylene blue (EMB) agar (Oxoid Ltd., Detroit, Michigan, USA) and incubated aerobically for 24 h at 37°C. Sterile loopful of characteristic colonies on EMB agar (metallic green sheen) and reddish/pinkish colonies on MAC agar was subjected to biochemical tests for identification of *E. coli* using standard methods [32]. All isolates identified as *E. coli* were inoculated and plated on blood agar and sorbitol MacConkey (SMAC) agar plates, which were again incubated aerobically at 37°C overnight. Phenotypic characteristics of *E. coli*, specifically mucoid appearance and haemolysis on blood agar plates and the ability to ferment sorbitol on SMAC agar as earlier described, [13] were observed. The O157 serotype was detected amongst 3 to 5 *E. coli* isolates per agar plate, with characteristic appearance on SMAC agar by the use of *E. coli* O157 antisera (Oxoid Ltd., Michigan, Ohio, USA) using the slide agglutination test.

To isolate *Campylobacter* spp., swabs of gastrointestinal contents were inoculated onto *Campylobacter* blood-free agar containing CCDA (charcoal cefoperazone deoxycholate agar) supplement (Oxoid Ltd., Basingstoke, Hampshire, England) and incubated for 48 h at 42°C in an atmosphere of 8% $CO_2$ in an incubator (Formo Scientific Marietta, Ohio, USA) to detect thermophilic *Campylobacter.* Colonies (3 to 5) showing typical appearance of *Campylobacter* on blood-free agar plates, specifically grayish with running appearance and nontranslucent, were gram-stained. Isolates that were gram-negative with sea gull or comma-shaped appearance were presumptively classified as *Campylobacter* spp. The procedure of Lior [33] was used to identify *Campylobacter* spp. and to classify the isolates as either *C. jejuni* or *C. coli.*

To isolate *Salmonella* spp., approximately 1 g of intestinal contents of rats was added to 9 ml of selective enrichment broths: selenite cystein (SC) and tetrathionate (TT) broths, thoroughly agitated in a vortex mixer and incubated

overnight at 42°C and 37°C, respectively. Enriched broths were then subcultured onto xylose lysine desoxycholate, XLD agar (Oxoid), and brilliant green agar (BGA) (Oxoid Limited, Detroit, Mich., USA) and incubated aerobically at 37°C and examined after 24 h. Suspect isolates (3 to 5) of *Salmonella* spp., which were pink colonies with black centers on XLD agar and pink colonies on BGA were subjected to biochemical tests using standard methods [32]. Biochemically identified *Salmonella* isolates were, thereafter, tested by slide agglutination using commercially available *Salmonella* polyvalent antiserum (Ai & Vi) (Difco Ltd., Detroit, Mich., USA). All isolates positive by the slide test were sent to the Caribbean Epidemiology Centre (CAREC), Port of Spain, Trinidad and Tobago, for confirmation and serotyping.

*2.6. Determination of Resistance to Antimicrobial Agents.* The resistance of isolates of *E. coli, Salmonella* spp. and *Campylobacter* spp. to the nine antimicrobial agents was determined using the disc diffusion method. The antimicrobial agents and the concentrations used were as follows: gentamicin (CN, 10 $\mu$g), ampicillin (AMP, 10 $\mu$g), cephalothin (KF, 30 $\mu$g), tetracycline (TE, 30 $\mu$g), streptomycin (S,10 $\mu$g), nalidixic acid (NA, 30 $\mu$g), kanamycin (K, 30 $\mu$g), chloramphenicol (C, 30 $\mu$g), and trimethoprim/sulpharmethoxazole (SXT, 23.25 $\mu$g, 1.75 $\mu$g). The breakpoints of the National Committee for Clinical Laboratory Standards [34] were used to determine susceptibility or resistance of isolates. For the study, all isolates that displayed resistance, based on their zone sizes, were classified as resistant isolates.

*2.7. Statistical Analyses.* The frequency of isolation of the three bacteria tested as well as the prevalence of resistance to the nine antimicrobial agents tested was compared and subjected to the chi-squared test ($\chi^2$). The level of significance was determined at an alpha level of 5%.

*2.8. Ethics Committee Approval.* Prior to the commencement of the study, the research proposal was approved by the Ethics Committee of the Faculty of Medial Sciences, University of the West Indies.

## 3. Results

*3.1. Geographical Locations of Trapped Rats.* Figure 1 displays the geographical locations across the country where rodents used in the study were trapped. Rats were trapped from a total of 44 geographical sites across 7 counties in the island. A majority of the rats sampled originated from the western part of the island, reflective of the fact that it was convenience sampling.

*3.2. Frequency of Isolation of Selected Bacteria.* Of a total of 204 trapped rats, intestinal contents were positive for *E. coli* (83.8%), *Campylobacter* spp. (3.4%), and *Salmonella* spp. (2.0%). The difference in the frequency of isolation was significant ($P < .05$; $\chi^2$) as shown in Table 1. Amongst the 7 isolates of *Campylobacter* spp., 3 were *C. jejuni* while 4 were *C. coli.* Of the 4 isolates of *Salmonella* spp. recovered, only 3 were typable and their serovars were as follows: Schwarzzund 4, 12: d: 1, 7, Senftenberg 1, 3, 19: g, (s), t, and Rubislaw 11: r: e, n, x.

Table 1: Frequency of isolation of *Escherichia coli*, *Campylobacter* spp., and *Salmonella* spp. from the faecal sample of rats.

| Type of bacteria | Number of samples tested | Number (%) of positive samples |
|---|---|---|
| *Escherichia coli* | 204 | 171 (83.8) |
| *Campylobacter* spp. | 204 | 7 (3.4)[a] |
| *Salmonella* spp. | 204 | 4 (2.0)[b] |

[a]Consisted of 4 (57.1%) isolates of *C. coli* and 3 (42.9%) isolates of *C. jejuni.*
[b]Comprised serovars *Schwarzengrund*, *Senftenberg*, *Rubislaw* and an untypable *Salmonella.*

Table 2: Characteristics of isolates of *Escherichia coli* from rats.

| Characteristic | Number of samples tested | Number (%) of positive samples |
|---|---|---|
| Mucoid | 171 | 25 (14.6) |
| Haemolytic | 171 | 0 (0.0) |
| Nonsorbitol fermenters | 171 | 19 (11.1) |
| O157 serotype | 171 | 0 (0.0) |

*3.3. Characteristics of Bacterial Isolates.* Table 2 shows the characteristics of *E. coli* isolates of which amongst the 171 isolates tested, 25 (14.6%) were mucoid and 19 (11.1%) were nonsorbitol fermenters (NSF) while all were negative for haemolytic or O157 strain of *E. coli.*

*3.4. Prevalence of Resistance of Bacteria to Antimicrobial Agents.* Overall, of 182 isolates of *E. coli, Campylobacter* spp., and *Salmonella* spp. tested, 71 (39.0%) exhibited resistance to one or more of the antimicrobial agents tested (Table 3). The prevalence of resistance was 36.3% (62 of 171), 75.0% (3 of 4), and 85.7% (6 of 7) for *E. coli*, *Salmonella* spp. and *Campylobacter* spp., respectively. The difference was statistically significant ($P < .05$; $\chi^2$). Amongst *E. coli* isolates, resistance to tetracycline (18.1%), ampicillin (15.8%), and chloramphenicol (8.2%) was higher compared with what was exhibited to cephalothin (0.0%), streptomycin (0.0%), and gentamicin (3.5%). Of the four *Salmonella isolates,* only one isolate was resistant to ampicillin, nalidixic acid, tetracycline, and chloramphenicol, and they were all susceptible to the five remaining antimicrobial agents tested. For the seven *Campylobacter* isolates, five exhibited resistance to cephalothin, sulphamethoxazole/Trimethoprim (SXT), streptomycin, and nalidixic acid while only one isolate was resistant to chloramphenicol and ampicillin.

## 4. Discussion

Rats are important as carriers and transmitters of a number of pathogens to humans and livestock as well as pet animals

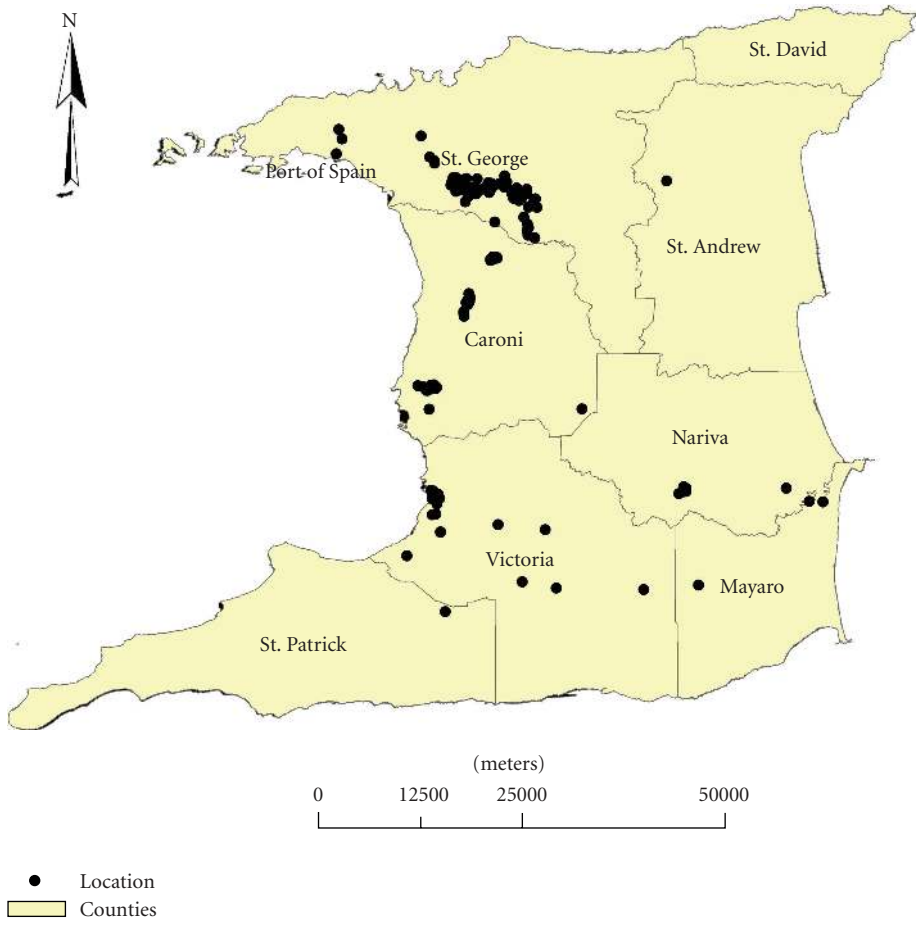


Figure 1: Location in Trinidad where rats were trapped for the study.

Table 3: Frequency of resistance to antimicrobial agents amongst zoonotic bacteria tested.

| Type of bacteria | Number of isolates tested | Number (%) of resistant isolates[a] | Number (%) of isolates resistant to: | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | KF[b] | CN | SXT | AMP | K | S | NA | TE | C |
| *Escherichia coli* | 171 | 62 (36.3) | 0 (0.0) | 6 (3.5) | 10 (5.8) | 27 (15.8) | 6 (3.5) | 0 (0.0) | 9 (5.3) | 31 (18.1) | 14 (8.2) |
| *Salmonella* spp. | 4 | 3 (75.0) | 0 (0.0) | 0 (0.0) | 0 (0.0) | 1 (25.0) | 0 (0.0) | 0 (0.0) | 1 (25.0) | 1 (25.0) | 1 (25.0) |
| *Campylobacter* spp. | 7 | 6 (85.7) | 5 (71.4) | 3 (42.9) | 5 (71.4) | 1 (14.3) | 4 (57.1) | 5 (71.4) | 5 (71.4) | 2 (28.6) | 1 (14.3) |
| Total | 182 | 71 (39.0) | 5 (2.7) | 9 (4.9) | 15 (8.2) | 29 (15.9) | 10 (5.5) | 5 (2.7) | 15 (8.2) | 34 (18.7) | 16 (8.8) |

[a] Resistant to one or more antimicrobial agents.
[b] KF-Cephalothin (30 $\mu$g), CN-Gentamicin (10 $\mu$g), SXT-Sulphamethoxazole/trimethoprim (23.25 $\mu$g, 1.75 $\mu$g), AMP-Ampicillin (10 $\mu$g), K-Kanamycin (30 $\mu$g), S-Streptomycin (10 $\mu$g), NA-Nalidixic acid (30 $\mu$g), TE-Tetracycline (30 $\mu$g), C-Chloramphenicol (30 $\mu$g).

thereby posing public health hazards to humans [5, 16, 35, 36]. It was therefore of epidemiological relevance that the rats trapped in the current study which were from as many as 44 locations across the island of Trinidad were positive for *E. coli*, *Salmonella* spp., and *Campylobacter* spp. which have the potential to be bacterial pathogens. Equally important is the fact that a majority of the rats were trapped in areas close to human habitation and market areas making contamination of human foods and environment a possibility.

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 *Enterobacteriaceae* in animals [37]. The prevalence of 83.8% found in rats in the current study is slightly higher than the 61.8% reported for rodents at the local zoo in the country [9]. It is, however, known that a majority of *E. coli* strains are commensals, but pathogenic or enterotoxigenic strains are known to exist [14, 37]. In mammalian wildlife that are free-ranging, 58% were positive for *E. coli* [8], for wildlife kept on private farms and at the zoo, the prevalence of *E. coli* in faecal materials was 88.2% [8] and 88.1% [9], respectively.

Of importance are the characteristics of the *E. coli* strains although most are commensals in the gastrointestinal tracts of animals [38]. Mucoid colonies and production of

haemolysins have been considered as virulence markers for *E. coli* strains [12]. In the current study, 14.2% of the isolates were mucoid, a frequency considerably higher than the 2% found in bats [10] and the 4.6% reported for mammalian wildlife at the local zoo [9]. None of the *E. coli* isolates produced haemolysin, a finding at variance with the report on isolates from other wildlife in the country where 10.2% were from bats [10], and 3.6% for mammalian wildlife at the local zoo were found to be haemolysin producers [9].

*E. coli* O157 serotype has become a very important food-borne pathogen globally because of the verocytotoxins they produce [14]. It has been demonstrated that most *E. coli* O157 serotypes are nonsorbitol fermenters [13] although some sorbitol-fermenting *E. coli* O157 strains have been reported [39]. In the current study, as many as 11.1% of the isolates were nonsorbitol fermenters, a finding higher than that found in *E. coli* isolates from free-ranging mammalian wildlife in the country where the frequency of nonsorbitol fermenting strains was 0.4% [8], wildlife on private farms 3.1% [8], and at a local zoo 3.0% [9]. All *E. coli* isolates (sorbitol and nonsorbitol fermenters) were, however, non-O157 serotype as earlier reported for *E. coli* strains recovered from wildlife sampled from various sources in the country [9]. This is a further evidence that wildlife in the country are not important reservoir for *E. coli* O157 or verocytotoxigenic *E. coli* (VTEC). It is, however, pertinent to mention that non-O157 Shiga toxin-producing *E. coli* has been documented in the literature [14]. Studies in other countries have, however, reported the isolation of *E. coli* O157 strain from rats and other wildlife, with the obvious potential that they could transmit this important pathogen to other animals and contaminate foods and the environment [40–42].

The prevalence of resistance (36.3%) exhibited by *E. coli* isolates from rats is considerably higher than the 20% found in rats in Kenya [43] but much lower than the 61.8% reported in Trinidad and Tobago [9]. Similarly, the prevalence of resistance, which by comparison to other antimicrobial agents, was high to tetracycline (18.1%), ampicillin (15.8%), and chloramphenicol (8.2%) but lower than the rates reported for in Trinidad. For example, *E. coli* isolates from free-ranging wildlife in the country had a prevalence of resistance of 37.2% to ampicillin but 1.3% to chloramphenicol [44] while for confined wildlife, the corresponding prevalence was 21.7% and 11.3% [9]. The observed low prevalence of resistance to gentamicin (3.5%), cephalothin (0.0%), and streptomycin (0.0%) is however in agreement with published reports on mammalian wildlife in the country by others [9, 44].

The frequency of 2.0% for *Salmonella* spp. in the current study is considerably lower than that found in other countries: 6.0% in France [45], 10.0% in the UK [29], 16.2% in the USA [46], and 32% in Nigeria [47]. Gopee et al. [26] had earlier reported 0% prevalence for *Salmonella* spp. in rats sampled at a zoo in the country.

The serotypes of *Salmonella* spp. isolated from rodents have been reported to be epidemiologically significant based on the fact that molecular studies established their association with human salmonellosis [17, 18, 36]. Although only three of the four isolates were typable, it is relevant to mention that these serotypes have been recovered from human gastroenteritis [48, 49], confined birds [26], pet dogs [50], and from captured bats [10] in the country.

The four isolates of *Salmonella* spp. in the current study exhibited resistance to ampicillin, nalidixic acid, tetracycline, and chloramphenicol. Although the number of isolates recovered was low, it has been reported that rodents served as sources of multiresistant *Salmonella* spp. in cases and epidemics of human salmonellosis [17, 18]. Resistance to antimicrobial agents has been reported by others [51] to reflect the use of antimicrobial agents in human and animal populations.

The frequency of isolation of 3.4% for *Campylobacter* spp. found in the 204 rats sampled in the country is low compared to the 18% prevalence reported for rats trapped in France [46] and 57.4% for black rats in Portugal [52]. A survey of other mammalian wildlife (free-ranging on land, confined or farmed, and in free-flying bats) in the country reported similarly low prevalence that ranged from 0% to 7.4% [10, 44, 53]. It, therefore, appears that the carriage rate for campylobacters in rats and wildlife is generally low in the country.

The resistance of isolates of *Campylobacter* spp. from rats in the current study was rather high, that is, five of seven resistant to cephalothin, SXT, streptomycin, and nalidixic acid but also low with one of seven isolates resistant to chloramphenicol and ampicillin. In a study on *Campylobacter* spp. isolated from wildlife including rats in Portugal, a frequency of resistance of 5.5% to ampicillin and tetracycline was reported [52]. It is, however, pertinent to mention that factors such as selected antimicrobial concentrations, methods, and the breakpoints used, affect the antibiograms obtained and should be considered in comparing antimicrobial resistance of bacteria in different studies.

It was concluded that because the rats sampled originated from locations across the country and were shown to be carriers of enteric pathogens *(Salmonella* spp. *Campylobacter* spp.), albeit at a low frequency, might have posed potential health risk to livestock, pet animals, and humans in the geographical location from where they were trapped. The possibility of them being carriers of other uncultured pathogens also cannot be ignored. It is therefore imperative that regular rodent control measures should be practiced to reduce this risk.

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