



Research Article

# Occurrence of Antibiotic Resistant *Escherichia Coli* in Green Iguanas (*Iguana Iguana*) in Grenada, West Indies

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## Abstract

Cloacal swabs from 62 green iguanas (*Iguana iguana*) from five parishes of Grenada were sampled during the period January to April 2013, and examined by culture for presence of *Escherichia coli*. Forty percent of the green iguanas were positive for *E. coli*. This organism is documented to cause health problems in wildlife species, but it is rare. Isolates were further tested for the presence of *E. coli* O157:H7, a serotype known to cause severe zoonotic illnesses globally. None of the isolates tested positive for this serotype. The results of this study indicate that green iguanas are not important reservoirs of *E. coli* O157:H7. Antimicrobial susceptibility tests conducted by a disk diffusion method against amoxicillin-clavulanic acid, ampicillin, cefotaxime, ceftazidime,

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ciprofloxacin, enrofloxacin, gentamicin, nalidixic acid, streptomycin, tetracycline and trimethoprim-sulfamethoxazole showed that the most frequent resistance was detected to amoxicillin-clavulanic acid and streptomycin. Isolates resistant to ampicillin, cefotaxime, tetracycline, and trimethoprim-sulfa were also detected. Overall, drug resistance was found for seven of the twelve antibiotics used in this study. Multiple drug resistance was found in 3 isolates. The antibiotic resistance patterns of *E. coli* found in this study is of public health concern since there are ample opportunities for transmission of enteric bacteria from iguanas to humans in Grenada. This is the first report of isolation of *E. coli* and antimicrobial resistance profiles from green iguanas in Grenada, West Indies.

**Keywords:** Iguana, *Escherichia coli*, drug resistance, Grenada

## Introduction

Non-pathogenic *Escherichia coli* constitute an important part of the normal intestinal microbiota of healthy mammals and birds whereas pathogenic strains may cause zoonotic infections in humans (Quinn *et al.*, 2011). *E. coli* has been responsible for both intestinal and extra-intestinal infections in humans (Santos *et al.*, 2013). In recent years, *E. coli* O157:H7 and other enterohemorrhagic serotypes have emerged as major food-borne, zoonotic pathogens in humans (Perna *et al.*, 2001, Quinn *et al.*, 2011).

Green iguanas are arboreal lizards that are native to the territories extending from Southern Mexico through to Central Brazil, Paraguay, Bolivia and the Galapagos islands (Taddei *et al.*, 2010). They can also be found in North America, Hawaii, Fiji and the Caribbean islands (Alberts *et al.*, 2004), including Grenada. Green iguanas constitute an important component of terrestrial and arboreal herpetofauna of many Caribbean islands (Alberts *et al.*, 2004). Green iguanas may act as sources of zoonotic pathogenic *E. coli*. The green iguana has been previously implicated as an important reservoir in the transmission of zoonotic pathogens to humans through contact with iguana feces (Mermin *et al.*, 1997). The increasing popularity of green iguanas as pets and as sources of meat for human consumption in Grenada, justifies the need to investigate their microbiological and zoonotic potential. The research hypothesis was that green iguanas could be carriers of *E. coli* O157:H7,

but drug resistance is unlikely. Based on this, the first objective of this study was to determine the occurrence of *E. coli* in the feces of green iguanas in Grenada. The second objective was to determine the prevalence of *E. coli* O157:H7 among the isolates. Determination of the resistance profiles of the *E. coli* isolates against antimicrobial drugs, commonly used in treating enteric infections in humans was the third and final objective of this study.

## Materials and Methods

During the period January to April 2013, a total of 62 cloacal swab samples were collected from wild and pet green iguanas and were analyzed for the presence of *E. coli*. Green iguanas were sampled in five of the seven parishes of Grenada, including from St. George 18, St. Andrew 10, St. David 10, St. Patrick 19, Carriacou and Petite Martinique 5. The wild iguanas were trapped and restrained using non-chemical humane traps and hand catching while the pet iguanas were safely retrieved from their respective cages with consent from their owners.

The trapped iguanas were examined by a veterinarian to ensure that they did not have any clinical signs of illness or injury. Only healthy green iguanas were included in this study. For each sampling event, a sterile transport swab (Starswab II™, Starplex™ scientific inc, Etobicoke, Ontario, Canada) was inserted two (2) inches into their rectum via the cloaca and gently rotated five times so as to obtain an adequate fecal sample. After sampling, the trapped and sampled iguanas

were tagged for identification purposes with regard to the location, time and date of sample collection using a permanent, non-toxic paint (VIBE Standard White, European Body Art Laboratory, Newport Beach, CA, USA). All the sampled iguanas were then returned to their respective natural habitats without subjection to transportation, diet change or excessive handling. The cloacal swabs were immediately stored in a cooler with ice packs and transported to the Bacteriology Laboratory, School of Veterinary Medicine, St. George's University where all the laboratory analysis were performed. The approximate time between sample collection and culture was three hours.

For the identification and isolation of *E. coli*, the cloacal swabs were placed in 10 ml of tryptic soy broth (Remel, Lenexa, KS, USA) and incubated at 37°C for 24 hours. After incubation, the swabs were streaked onto MacConkey agar and incubated at 37°C for 24 hours. One to two (1 – 2) pink to red color colonies with or without a zone of precipitated bile morphologically resembling *E. coli* were subcultured via streaking onto individual MacConkey agar and incubated at 37°C for 24 hours. The pure colonies from second MacConkey agar were further tested by using API-20E® (Analytical profile Index; Bio-Merieux Inc., Durham, NC, USA) strips for confirmation as *E. coli*.

For identification of *E. coli* O157:H7 pure colonies were first plated on sorbitol-MacConkey agar and then tested using Remel Wellcolex\* *E. coli* O157 Rapid Latex Test (Remel Europe Ltd; Clipper Boulevard West, Crossways, Dartford, Kent, DA2 6PT, UK) and Prolex™ *E. coli* O157 Latex Kit (Pro-lab

Diagnostics, 20 Mural Street, Units 3 & 4, Richmond Hill, Toronto, Canada).

The antimicrobial susceptibility tests were carried out using the disk diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) using Mueller Hinton agar, and the inhibition zone sizes were interpreted as per CLSI guidelines (Jorgensen and Turnidge, 2003). The antibiotic disks used were amoxicillin/clavulanic acid, ampicillin, cefotaxime, ceftazidime, ciprofloxacin, enrofloxacin, gentamicin, imipenem, nalidixic acid, streptomycin, tetracycline and trimethoprim/sulfamethoxazole (Becton, Dickinson and Co., Sparks, MD, USA).

## Results

In this study, *E. coli* was isolated only from 25 (40%) of the 62 green iguanas (Table 1). Based on gender, 43% (15 of 35) females and 36% (10 of 27) male iguanas tested positive for *E. coli*. The percentages of iguanas that tested positive for *E. coli* based on habitat, sex, life stage and parishes are summarized in Table 1. No statistical difference was found between male and female iguanas ( $p=0.64$ ), between pet and wild iguanas ( $p=0.98$ ) and between adult and young iguanas ( $p=0.71$ ) in our study, however, significant statistical difference was found among the five parishes from which iguanas were sampled ( $p=0.003$ ) using Chi-square test at a 95% confidence interval (Table 1). It may be noted that this may be a trend since the number of iguanas sampled from each parish varied as outlined in the methodology. A total of 42 *E. coli* isolates were obtained from the 25 culture-positive iguanas, all confirmed by API-20E identification system.

**Table 1: Statistical Analysis for *E. coli* Prevalence in Green Iguanas from Grenada.**

	Number of iguanas sampled	Positive for <i>E. coli</i> (%)	Chi-square	p-value
Prevalence	62	25 (40)		
Gender			0.22	0.64
Female	35	15 (43)		
Male	27	10 (37)		
Life Stage			0.14	0.71
Adult	43	18 (42)		
Young	19	7 (37)		
Parish			16.31	0.003
St. Andrew's	10	2 (20)		
Carriacou	5	3 (60)		
St. David's	10	1 (10)		
St. George's	18	5 (28)		
St. Patrick's	19	14 (74)		
Habitat			0.001	0.98
Pet	15	6 (40)		
Wildlife	47	19 (40)		

With regard to identification of *E. coli* O157:H7 among the positive isolates; 12 of the 42 (29%) *E. coli* isolates tested negative for sorbitol fermentation on sorbitol-MacConkey agar, however, none of the 42 *E. coli* isolates tested positive for *E. coli* O157:H7 using Remel Wellcolex\* *E. coli* O157 Rapid Latex Test and Prolex™ *E. coli* O157 Latex.

Based on the result of the disk diffusion assays, five (12%) of the isolates recovered from the positive iguanas were resistant to

amoxicillin/clavulanic acid and to streptomycin, 3 (7%) were resistant to ampicillin and 1(2%) each to nalidixic acid, cefotaxime, tetracycline and trimethoprim/sulfamethoxazole. Seven (17%) showed intermediate resistance to ampicillin, 6 (14%) to streptomycin, 1 (2%) to tetracycline, cefotaxime, and ceftazidime. Three isolates showed multiple drug resistance. The antibiotic susceptibility profiles of the 42 *E. coli* isolates recovered from the positive iguanas are presented in Table 2.

**Table 2: Antimicrobial Susceptibility Profiles of 42 *E. coli* Isolates from Green Iguanas in Grenada Using Disk Diffusion Method.**

Antimicrobial Agent	Conc. (µg)	R	I	S
		No. (%)	No. (%)	No. (%)
Amoxicillin/clavulanate (AmC)	30	5 (12)	0 (0)	37 (88)
Ampicillin (AM)	10	3 (7)	7 (17)	32 (76)
Cefotaxime (CTX)	30	1 (2)	1 (2)	40 (96)
Ceftazidime (CAZ)	30	0 (0)	1 (2)	41 (98)
Ciprofloxacin (CIP)	5	0 (0)	0 (0)	42 (100)
Enrofloxacin (ENO)	5	0 (0)	0 (0)	42 (100)
Gentamicin (GM)	10	0 (0)	0 (0)	42 (100)
Nalidixic acid (NA)	30	1 (2)	0 (0)	41 (100)
Streptomycin (S)	10	5 (12)	6 (14)	31 (74)
Trimethoprim/sulfamethoxazole (SXT)	1.25 / 23.75	1 (2)	0 (0)	41 (98)
Tetracycline (Te)	30	1 (2)	1 (2)	40 (96)
Imipenem (IPM)	10	0 (0)	0 (0)	42 (100)

<sup>1</sup>Three isolates showed multiple drug resistance. The resistance patterns of the three isolates were: (1): AmC+AM+S+SXT+Te (2): AmC+S, and (3): AmC+AM.

## Discussion

The isolation rate for *E. coli* (40%) in the current study is somewhat similar to that reported in captive green iguanas in Trinidad and Tobago where 60% captive green iguanas tested positive for *E. coli*. That same study showed that 58% of free-ranging mammals yielded *E. coli* and 90% of free-flying birds were positive for *E. coli* (Adesiyun, 1999). In a study conducted in Australia on non-mammalian vertebrates, 33% of crocodiles, 4% of turtles, 2% of snakes and 10% of lizards tested positive for *E. coli* (Gordon and Cowling, 2003). *E. coli* is documented to cause health problems in wildlife species, but this is considered rare (Adesiyun, 1999). *E. coli* has been reported as a cause of diarrhea in a tortoise (Owuamanam et al. 2012). Free-flying birds and free-ranging mammals harbor more *E. coli* in their gastrointestinal tract than reptiles do (Adesiyun, 1999).

Furthermore, 29% of the *E. coli* isolates in the present study were negative for sorbitol fermentation on sorbitol-MacConkey agar. *E. coli* O157:H7 is typically a non-sorbitol fermenter, however other zoonotic

pathogenic non-O157 strains of *E. coli* are also non-sorbitol fermenters. Some pathogenic non-O157 strains of *E. coli* including O26, O103 and O111, have been previously associated with infections in humans. There is a possibility that there are other pathogenic Shiga-toxin producing *E. coli* strains among the isolates, however this study focused on *E. coli* O157:H7 since it is of paramount public health importance (Adesiyun, 1999). Additionally, the most commonly isolated *E. coli* serotype implicated in hemorrhagic colitis disease outbreaks in humans globally is *E. coli* O157:H7 and it serves as a marker for virulent strains of *E. coli* in various species (Nataro and Kaper, 1998, Perna et al., 2001).

*E. coli* O157:H7 was not identified in this study using Latex Agglutination Test Kits. In Trinidad and Tobago, all samples from 5 green iguanas fermented sorbitol and also tested negative for *E. coli* O157:H7 on Latex Agglutination Test. Free-ranging and captive avian, reptile and mammalian wildlife species were not important reservoirs of *E. coli* O157:H7 in Trinidad and Tobago (Adesiyun, 1999). Similarly, it appears that

green iguanas in Grenada do not harbor *E. coli* O157:H7 and may therefore not pose a public health in this regard. However, it may be noted that other methods of detecting *E. coli* O157:H7, including PCR are available but they were not employed in this study.

Although there is no information on Shiga-toxin producing *E. coli* in green iguanas in England, wild rabbits appear to be significant reservoirs of *E. coli* O157:H7 in that country; 16.2% fecal samples from wild rabbits tested positive for Shiga-toxin producing *E. coli* and 6.2% of said fecal samples were confirmed as *E. coli* O157:H7 (Sciafe and Crook, 2005). In a study in the United States, using culture, latex agglutination and PCR, 521 fecal samples from wild life species including raccoons (230 samples), deer (141 samples), opossums (25 samples), birds (9 samples), and other species (16 samples) were analyzed; only one (0.19%) wild opossum of the 521 samples tested positive for *E. coli* O157 (Renter *et al.*, 2003). *E. coli* O157:H7 was isolated from 23.4% feral pigs in California, USA. This study confirmed that mammalian wild life species in California, USA may be implicated in cross contamination of vegetables destined for human consumption and therefore constitute an important public health source of pathogenic *E. coli* (Jay-Russell, 2010). In another study conducted in three counties in California, USA, 730 samples from various wild life species including raccoons, opossums, striped skunk and tule elk tested negative for *E. coli* O157:H7 (Mandrell *et al.*, 2010). Obviously, wildlife species other than rabbits and feral pigs are not common reservoirs of this serotype of Shiga-toxin producing *E. coli* (Renter *et al.*, 2003).

Green iguanas have however been shown to be significant sources of zoonotic pathogenic *Salmonella* in Grenada (Sylvester *et al.*, 2013). It is possible that in the current study the prevalence of *E. coli* O157:H7 is underestimated since PCR and/or serotyping of *E. coli* were not performed. Due to the frequent human-wildlife interactions, the

proximity of human and wildlife habitats as well as the tendency for Grenadians to consume wild meat, there is an urgent need to conduct continued research in wildlife species in Grenada. Future studies should include use of PCR and serotyping.

In this current study antibiotic resistance was detected for seven of the twelve antibiotics used. The highest resistance was 12%, recorded for amoxicillin/clavulanic acid and streptomycin, followed by ampicillin and nalidixic acid, cefotaxime, tetracycline and trimethoprim/sulfamethoxazole. It is interesting to note that resistance was found in multiple antimicrobial drug categories/classes including penicillins, tetracyclines, cephalosporins, aminoglycosides, sulfanomide-trimethoprim and quinolones (Table 2). Additionally, it should be considered significant that one *E. coli* isolate showed intermediate resistance to two third generation cephalosporins (cefotaxime and ceftazidime) used in treating complicated systemic bacterial infections (Pournaras *et al.*, 2004). This antibiotic resistance pattern differs to that reported in *Salmonella* isolates from green iguanas, blue land crabs and cane toads in Grenada where no resistance was reported (Sylvester *et al.*, 2013; Peterson *et al.*, 2013; Drake *et al.*, 2012). In a study of 54 *E. coli* isolates from feral cats in Grenada, no resistance was found in 65% of the isolates tested against 6 drugs. Resistance to amoxicillin-clavulanic acid was found only in 2% of the isolates (Hariharan *et al.*, 2011). The relatively high antibiotic resistance amongst *E. coli* isolates from green iguanas is of public health concern since these resistance genes may be transferred to other non-pathogenic and/or pathogenic *E. coli* and other members of *Enterobacteriaceae*, including *Salmonella* in the environment or in the human gut. We propose that future studies on *E. coli* in wildlife should include determination of minimal inhibitory concentrations of antimicrobial drugs for critical analysis of drug resistance.

## Conclusions

In this study *E. coli* O157:H7 was not detected in green iguanas in Grenada. The antibiotic resistance patterns found in this study are of public health concern since there are ample opportunities for transmission of pathogenic and/or commensal microorganisms from iguanas to humans, particularly because green iguanas are used for human consumption in Grenada.

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