Antimicrobial resistance patterns of commensal \textit{Escherichia coli} isolated from feces of non-diarrheic dogs in Grenada, West Indies

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Received: 29-06-2019, Accepted: 25-11-2019, Published online: 27-12-2019

doi: www.doi.org/10.14202/vetworld.2019.2070-2075  How to cite this article: Amadi VA, Hariharan H, Amadi OA, Matthew-Belmar V, Nicholas-Thomas R, Perea ML, Carter K, Rennie E, Kalasi K, Alhassan A, Kabusuu RM, Alozie GU, Fields PJ, Pinckney R, Sharma R (2019) Antimicrobial resistance patterns of commensal \textit{Escherichia coli} isolated from feces of non-diarrheic dogs in Grenada, West Indies, Veterinary World, 12(12): 2070-2075.

Abstract

Background and Aim: There is currently no published information on the prevalence and antimicrobial susceptibility patterns of commensal \textit{Escherichia coli} in dogs of Grenada origin. Monitoring antimicrobial resistance helps in the empirical selection of antibiotics. This study determined the occurrence of \textit{E. coli} including the O157:H7 serotype in feces of non-diarrheic dogs of Grenada origin and the antibiotic resistance pattern of the \textit{E. coli} isolates.

Materials and Methods: Fecal samples from 142 of the 144 (98.6\%) dogs were culture positive for \textit{E. coli}. Selection of up to three colonies from each of the 142 \textit{E. coli}-positive samples yielded a total of 402 \textit{E. coli} isolates, which were analyzed for the presence of non-sorbitol fermenting colonies, and O157-agglutination.

Results: Of the 402 \textit{E. coli} isolates, 30 (7.5\%) were non-sorbitol fermenters. However, none of the 402 isolates gave a positive reaction (O157:H7) to the \textit{E. coli} O157:H7 latex kit. Antimicrobial susceptibility tests against 12 antibiotics revealed a resistance rate to the tested antibiotics except for tetracycline (Te) (23.4\%), cephalothin (CF) (13.2\%), and ampicillin (AM) (7.7\%). Thirty-nine out of the 402 (9.7\%) isolates were resistant to two or more antibiotics of different classes.

Conclusion: This is the first report of isolation and antimicrobial susceptibilities of commensal \textit{E. coli} from non-diarrheic dogs in Grenada. Some of the isolates (39/402 isolates, 9.7\%) were resistant to multiple antibiotics. This study showed that presently, dogs in Grenada should not be considered a reservoir for the \textit{E. coli} O157:H7 serotype and for multiple antibiotic-resistant \textit{E. coli} strains. Among the 402 \textit{E. coli} isolates, the resistance rate to drugs other than Te, CF, and AM was very low.

Keywords: antimicrobial resistance, commensal \textit{Escherichia coli}, dogs, Grenada.

Introduction

\textit{Escherichia coli} is commonly found in the intestinal flora of humans and other mammals [1-3], including dogs. \textit{E. coli} is excreted in feces and can be easily spread through food, water, and soil [1,2]. Most \textit{E. coli} strains are non-pathogenic [4], however, some strains represent primary pathogens having the potential to cause diseases, especially diarrhea in humans and animals [5,6], suggesting zoonotic transmission [7].

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and animals [15]. There have been no published reports on the prevalence and antimicrobial susceptibility of *E. coli* including *E. coli* O157:H7 serotype in dogs in Grenada. However, several Grenada studies have shown that antibiotic resistance is minimal among different bacteria isolated from other animals, including wild and domestic animals, and crustaceans, and tetracycline (Te) resistance is common in Grenada [3,16-25].

The objectives of the present study were to determine the occurrence of *E. coli*, including the O157:H7 serotype in feces of non-diarrheic dogs of Grenada origin, and to study the antibiotic resistance pattern for the *E. coli* isolates.

**Materials and Methods**

**Ethical approval**

The St. George’s University Institutional Animal Care and Use Committee approved the study (IACUC 15006-R).

**Sample collection**

The method used for the collection of samples has been previously described [17]. Briefly, the tested dogs were sampled from August to October 2016, the dog owners signed consent form to enter their dogs in the study, and the dogs were randomly selected from six of the seven parishes of the island of Grenada: St. George’s Parish 32, St. David’s Parish 28, St. Andrew’s Parish 26, St. Patrick’s Parish 24, St. Mark’s Parish 16, and St. John’s Parish 18 [17]. The gender, age, housing (indoor/outdoor or strictly indoor), breed, health history, history of antibiotic use, and date of sampling of the tested dogs were recorded [17]. Approximately 1-2 g of fresh fecal sample was collected from each dog [17,26], placed in a vial of transport medium and placed in a cooler with ice packs, and transported to the Bacteriology Laboratory, School of Veterinary Medicine, St. George’s University for laboratory analysis [17].

**Isolation and identification of *E. coli***

For the isolation of *E. coli*, the culture methods previously described [3,16,20] were used with slight modification. The contents of each vial were mixed and an aliquot (1 ml) was transferred into a tube containing 10 ml of tryptic soy broth (Remel, Lenexa, KS, USA) and incubated at 37°C for 18-24 h, as previously described [17]. After incubation, an aliquot was then subcultured onto MacConkey agar (MAC) (Remel, Lenexa, KS, USA) and incubated at 37°C for 24 h [3,16,20]. To increase the possibility of identifying *E. coli* O157:H7 in a sample, three pink to red color colonies with or without a zone of precipitated bile morphologically representing *E. coli* were subcultured onto individual MAC agar and incubated at 37°C for 24 h for isolation of pure colonies. The pure colonies were identification and confirmation of the colonies as *E. coli* using the analytical profile index strips (API20E-BioMérieux, Hazelwood, MO) [3,16,20]. Atypical isolates identified as *E. coli* by API20E were also added in the study, as previously described [3,16,20].

**Identification of *E. coli* O157:H7 serotype**

The identification of *E. coli* O157:H7 was performed using the methods, as previously described [3,16,20]. Briefly, the pure colonies were plated on sorbitol-MAC (Remel, Lenexa, KS, USA) and incubated at 37°C for 24 h. After incubation, the sorbitol-MAC plates were examined for the presence of non-sorbitol fermenting colonies. All the colonies (both the sorbitol and the non-sorbitol fermenting colonies) were then subjected to slide agglutination using *E. coli* O157:H7 latex kit – Remel Wellcolex × *E. coli* O157 rapid latex test (Remel Europe Ltd., Kent, UK). The *E. coli* isolates that showed negative reaction to the latex test kit were considered to be *E. coli* O157:H7 negative [3,16,20].

**Antimicrobial susceptibility test**

Antimicrobial susceptibility testing was performed, as previously described [3,16,20] following the Clinical and Laboratory Standards Institute (CLSI) performance standards [27]. All the *E. coli* isolates were tested for susceptibility to the 12 antibiotics used in our previous study [17]. The antibiotic disks used were (BD BBL™ Sensi Disk™): Amoxicillin-clavulanic acid (AmC-20/10 µg), ampicillin (AM-10 µg), cefotaxime (CTX-30 µg), ceftazidime (CAZ-30 µg), cefalothin (CF-30 µg), chloramphenicol (C-30 µg), ciprofloxacin (CIP-5 µg), gentamicin (GM-10 µg), imipenem (IPM-10 µg), neomycin (N-30 µg), Te (Te-30 µg), and trimethoprim-sulfamethoxazole (SXT-1.25/23.75 µg) [17]. The inhibition zone sizes were interpreted based on CLSI guidelines [28] as recommended by the disk manufacturer (BD BBL™ Sensi Disk™). *E. coli* ATCC 25922 was used as a quality control strain [28].

**Statistical analysis**

Online data analysis software: http://www.openepi.com/Menu/OE_Menu.htm was used for all the statistical analyses [17]. The OpenEpi – two by two table (Chi-squared analysis) was used to compare the differences in the proportions of female versus male dogs, indoor/outdoor dogs versus strictly indoor dogs, and <1 year versus >1 year dogs that were culture-positive for *E. coli* while the z-test analysis was used to compare the proportion of *E. coli* isolates showing single antibiotic resistance pattern versus those showing multiple antibiotic resistance pattern. The level of statistical significance was set at α=0.05, p<0.05 was considered statistically significant.

**Results**

One hundred and forty-four non-diarrheic owned dogs were enrolled in the study. They comprised 140 (97%) indoor/outdoor dogs and four (3%) strictly indoor dogs. By gender, 56 (39%) were female and 88 (61%) were male, and by age, 46 (32%) were <1 year (<1), and 98 (68%) were >1 year (>1). All the tested dogs were mixed breed dogs known colloquially as
isolates that were strains 0
0
381 (94.8)
399 (99.3)
394 (98)
0
6 (1.5)
O157:H7 [29].
O157:H7 in dogs and cats showed that only 8 (2)
14 (3.5)
1 (0.2)
357 (88.8)
152 (37.8)
1 (0.2)
395 (98.3)
399 (99.3)
0
2 (0.5)
(30)
53 (13.2)
197 (49)
152 (37.8)
6 (1.5)
1 (0.2)
395 (98.3)
399 (99.3)
2 (0.5)
0
2 (0.5)
0
2 (0.5)
0
3 (0.7)
95 (23.6)
304 (75.6)
26 (23.4%) followed by CF (13.2%) (Table-1). Ten of
the tested dogs had received antibiotics 2 weeks before
sampling. Antimicrobial-resistant E. coli strains
were isolated from seven out of the ten dogs. The
most common resistance seen among the dogs was
to Te, of which isolated from six of the seven dogs
showed resistance to Te. Some of the E. coli isolates
(39/402 isolates, 9.7%) were resistant to multiple anti-
biotics (multidrug resistance [MDR] to two or more
antibiotics of different classes) (Table-2), while some
(113/402 isolates, 28.1%) showed resistance to a sin-
gle antibiotic. The isolates showing MDR were sig-
nificantly fewer than those showing resistance to one
antibiotic (p<0.001). The most common MDR pattern
observed was to three antibiotics (AM/SXT/Te), of
which eight E. coli isolates showed the pattern. Six iso-
lates showed MDR to four antibiotics (AM/C/SXT/Te)
and two other isolates also showed MDR to four anti-
biotics (AM/CF/SXT/Te) (Table-2).

Discussion
In this study, E. coli was isolated from 142/144 (98.6%)
dogs. The results of this study indicate that presently, non-diarrheic dogs in Grenada harbor E. coli in their gastrointestinal tracts and the occurrence is widespread among dogs in Grenada irrespective of their distribution, gender, housing, breed, health history, and history of antibiotic use. It is pos-
sible that the two culture-negative dogs in this study
may still be sub-clinical shedders of E. coli because
fecal shedding can be dynamic.

Our study showed a high prevalence rate of E. coli in non-diarrheic dogs in Grenada, none of the
tested dogs were positive for E. coli O157:H7 sero-
type based on our agglutination test results. In Japan,
a 3-year (1996-1998) epidemiological surveillance of E. coli O157:H7 in dogs and cats showed that only
one dog kept by a human patient infected with E. coli
O157:H7 tested positive for E. coli O157:H7 [29].
The authors reported that companion animals may not
give harbor to E. coli O157:H7 [29]. In the present
study, 30 (7.5%) of the E. coli isolates were non-sorbi-
tol fermenters that gave a negative reaction (no O157-
agglutinating) to the E. coli O157:H7 latex kits. This
study was designed to target only the E. coli O157:H7
serotype, which is usually non-sorbitol fermenters
that give a positive reaction to the E. coli O157:H7
latex kits. Hence, the 30 E. coli isolates that were
non-sorbitol fermenters that gave a negative reaction
to the E. coli O157:H7 latex kits were not identified in
relation to their serotypes.

| Antibiotic (disk concentration [μg]) | Resistant n (%)* | Intermediate n (%)* | Susceptible n (%)* |
|-------------------------------------|------------------|---------------------|-------------------|
| Amoxicillin-clavulanic acid (30)    | 2 (0.5)          | 8 (2)               | 392 (97.5)        |
| Ampicillin (10)                     | 31 (7.7)         | 14 (3.5)            | 357 (88.8)        |
| Cefotaxime (30)                     | 0                | 2 (0.5)             | 400 (99.5)        |
| Ceftazidime (30)                    | 2 (0.5)          | 0                   | 400 (99.5)        |
| Cephalothin (30)                    | 53 (13.2)        | 197 (49)            | 152 (37.8)        |
| Chloramphenicol (30)                | 6 (1.5)          | 1 (0.2)             | 395 (98.3)        |
| Ciprofloxacin (5)                   | 3 (0.7)          | 0                   | 399 (99.3)        |
| Gentamicin (10)                     | 6 (1.5)          | 2 (0.5)             | 394 (98)          |
| Imipenem (10)                       | 0                | 0                   | 402 (100)         |
| Neomycin (30)                       | 3 (0.7)          | 95 (23.6)           | 304 (75.6)        |
| Tetracycline (30)                   | 94 (23.4)        | 13 (3.2)            | 295 (73.4)        |
| Trimethoprim-sulfamethoxazole (1.25, 23.75) | 20 (5)          | 1 (0.2)             | 381 (94.8)        |

*#=Number, % (percentage)=Values are rounded up and down to one decimal place

Veterinary World, EISSN: 2231-0916 2072

Available at www.veterinaryworld.org/Vol.12/December-2019/25.pdf
Table-2: MDR patterns of the 39 *Escherichia coli* isolates from feces of non-diarrheic owned dogs in Grenada.

| Multiple antibiotic resistance pattern | Number of antibiotics to which the isolates are resistant | Number of isolates showing MDR (%) |
|----------------------------------------|-----------------------------------------------------------|-----------------------------------|
| GM/Te                                  | 5 (1.2)                                                   |                                   |
| N/Te                                   | 2 (0.5)                                                   |                                   |
| AM/Te                                  | 7 (1.7)                                                   |                                   |
| SXT/Te                                 | 1 (0.25)                                                  |                                   |
| CF/Te                                  | 3 (0.75)                                                  |                                   |
| CF/AM                                  | 1 (0.25)                                                  |                                   |
| AmC/CF                                 | 1 (0.25)                                                  |                                   |
| AM/GM/Te                               | 1 (0.25)                                                  |                                   |
| AM/SXT/Te                              | 8 (2)                                                     |                                   |
| N/SXT/Te                               | 1 (0.25)                                                  |                                   |
| AM/CF/Te                               | 1 (0.25)                                                  |                                   |
| AM/C/SXT/Te                            | 6 (1.5)                                                   |                                   |
| AM/CF/SXT/Te                           | 2 (0.5)                                                   |                                   |

% (percentage)=Values are rounded up and down to one decimal place. AmC=Amoxicillin-clavulanic acid, AM=Ampicillin, CTX=Cefotaxime, CAZ=Ceftazidime, CF=Cephalothin, C=Chloramphenicol, CIP=Ciprofloxacin, GM=Gentamicin, IPM=Imipenem, N=Neomycin, Te=Tetracycline, SXT=Trimethoprim-sulfamethoxazole, MDR=Multidrug resistance (resistance to two or more antibiotics of different classes)

Companion animals represent potential sources of spread of antimicrobial resistance due to the extensive use of antimicrobial agents in these animals and their close contact with humans [30]. Commensal *E. coli* in the intestines of animals, including dogs, can develop antibiotic resistance on exposure to antimicrobial agents. Considering the close contact that humans have with dogs, the high levels of antibiotic-resistant *E. coli* in canine feces may be a potential source of antibiotic-resistant bacteria or resistance determinants [31,32]. In the present study, the most prevalent resistance rate observed was for Te (23.4%) followed by CF (13.2%). In this study, 10% of the tested dogs had received antibiotics (eight received doxycycline, one received cephalaxin, and one amoxicillin) 2 weeks before sampling. The most common resistance seen among these dogs was resistance to Te. It is important to note that the household use of antibiotics by individuals in the homes where the dogs lived were not determined in this study. Antimicrobial agents are used therapeutically in humans and animals for control of bacterial infections [33]. It is possible that the resistant *E. coli* may have been acquired by the dogs through direct exposure to the individuals in the homes where they lived. Further study is needed to elucidate the role of individuals (in the homes where the dogs lived) in the transmission of the resistant *E. coli* strains. In Grenada, Te resistance is high, several studies have shown that Te resistance is common among a variety of Gram-negative bacteria from different sources [3,16-25]. It is noteworthy that chlorotetracycline is routinely used as a feed additive for food animals such as pigs in Grenada. Furthermore, oxytetracycline is used for the treatment of pigs for bacterial infections [1]. The food animals can easily contaminate human dwellings, fields, vegetables, and water sources with their feces. This, along with the environmental robustness of *E. coli* and large dog populations, may lead to long-term human and animal exposure to Te-resistant bacteria. In this study, two isolates were resistant to CAZ. These two CAZ-resistant isolates were also intermediate resistant to CF. It is noteworthy that the two CAZ-resistant isolates may be producing the extended-spectrum beta-lactamases or AmpC β-lactamases, although the current study was not designed to determine the resistance mechanisms involved.

In the present study, 9.7% of isolates showed MDR. In a recent study in the UK, 13.1% of fecal samples from hospitalized companion animals, 93% being dogs, yielded MDR *E. coli* [34]. In other studies, 15% of community-based dogs in the UK [35] and 32% of stray dogs in Korea [36] were positive for MDR. Resistance can develop by several mechanisms. Resistance to Te was the highest (23%) among all antibiotics used in this study, and it was characterized by MDR in 95% of 39 isolates in this study. Beta-lactam resistance was seen in 77% of MDR isolates in this study. *E. coli* is the predominant bacterial species associated with urinary tract infection (UTI) in dogs. MDR *E. coli* can be a problem in cases of UTI in dogs [37]. Emerging resistance to extended-spectrum cephalosporins including CAZ is of concern. *E. coli* strains from pet dogs and owners have been shown to have similar resistance patterns and genetic relatedness [38].

**Conclusion**

We documented the prevalence of *E. coli* in the feces of non-diarrheic owned dogs in Grenada to be 98.6%. This current study showed that presently, dogs in Grenada should not be considered reservoirs of *E. coli* O157:H7 serotype. Among the commensal isolates, resistance rates to drugs other than Te, CF, and AM were very low. Resistance to two or more antibiotics was seen only in 9.7% of the *E. coli* isolates. This indicates that non-diarrheic dogs in Grenada are presently not main reservoirs for multiple resistant *E. coli* strains.

**Authors’ Contributions**

VAA and HH designed the protocol and were involved with sample collection. MLP, KC, ER, KK,
and GUA were involved with sample collection. VAA, VM, RN, KK, and AA carried out all the laboratory work. VAA, RMK, and PJF performed data analysis and supervised the study. VAA wrote the paper, and OAA, HH, RP, RS were involved in the drafting of the manuscript. All authors revised, read, and approved the final manuscript.

Acknowledgments

This study was funded by the St. George’s University Small Research Grant Initiative (GSP/ SRGI/16002). The authors gratefully acknowledge the assistance of the clinicians and technicians at St. George’s University, Small Animal Clinic, and Ms. Jennifer Allen for their assistance with sample collection.

Competing Interests

The authors declare that they have no competing interests.

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