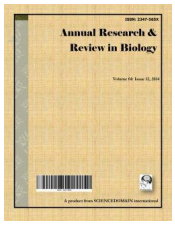

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Antimicrobial Drug Resistance in *Escherichia coli* Including an O157:H7 Isolate from Feces of Healthy Goats in Grenada

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Authors' contributions

This work was carried out in collaboration between all authors. Authors VAA, RS and HH designed the study. Authors RS and SG managed the collection of all the samples. Authors VAA, EA, OAO and ZP managed the analyses and literature searches. Authors VAA and HH wrote the protocol and wrote the first and final drafts of the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine: the occurrence of *Escherichia coli* including the O157:H7 serotype in feces of healthy goats in 10 randomly selected farms in Grenada and the antimicrobial drug resistance in *E. coli* isolated from feces of the tested goats.
Study Design: During the period of May to July, 2014, fecal samples were obtained from randomly selected healthy goats in 10 farms in Grenada and analyzed in the bacteriology laboratory, in the Pathobiology Department, School of Veterinary Medicine, St. George's University, Grenada.

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Methodology: Fecal samples were obtained from 70 randomly selected healthy goats in 10 farms in Grenada and cultured for *E. coli* and tested for O157:H7 serotype by the presence of non-sorbitol fermenting colonies and a positive reaction to O157-agglutination latex kits.
Results: All the 70 tested goats were culture positive for *Escherichia coli*. A total of 140 *E. coli* isolates were recovered and analyzed for the presence of non-sorbitol fermenting colonies and O157-agglutination. Of the 140 yielded *E. coli*, 11 (8%) isolates were non-sorbitol fermenters but only one (<1%) out of the non-sorbitol fermenters gave a positive reaction to the two *E. coli* O157:H7 latex kits. Antimicrobial susceptibility tests against 12 drugs showed susceptibility of the single *E. coli* O157:H7 isolate recovered to all the tested antibiotics. Among the non-O157:H7 isolates, the susceptibility rates to amoxicillin-clavulanic acid, ciprofloxacin, enrofloxacin, gentamicin, cefepime and ceftazidime ranged from 99% to 100%. The resistance rate to ampicillin, gentamicin, nalidixic acid, streptomycin, trimethoprim-sulfamethoxazole, and tetracycline observed was very low except to streptomycin (19%). Resistance to two or more antibiotics was observed only in 5% of the 140 *E. coli* isolates.
Conclusion: Our study revealed that presently, healthy goats in Grenada are not major reservoirs for the *E. coli* O157:H7 serotype and for multiple resistant *E. coli* strains.

Keywords: Escherichia coli; O157; goats; drug resistance; Grenada.

1. INTRODUCTION

Grenada which is approximately 344 km^2 in size is a volcanic island in the south Caribbean [1]. Kumthekar et al. [2] indicated that small ruminants are important production animals in Grenada due to the controllable size and feed requirements as well as their ability to utilize sloped landscape and limited pasture area. According to these authors, Grenada has an estimate small ruminant population of 2,500 goats, and the pasture land suitable for grazing livestock totals approximately 11,655 hectares of the Caribbean tri-island nation. Meat and milk from sheep and goats are commonly exploited for food and sold for as a source of income for many individuals in Grenada. In spite of the importance of goats, little is known about the prevalence and characteristics of bacteria that are associated with goats in Grenada.

E. coli is a common inhabitant of the large and lower small intestines of variety of mammals [3-5] including goats. It is excreted in feces and can be easily spread via soil, food and water [3,4]. A large number of *E. coli* strains are non-pathogenic [6], however, the pathogenic strains may cause severe intestinal or extra intestinal disease in humans [7] and are also capable of causing zoonotic infections [8]. Globally, more than 100 million cases of gastrointestinal illnesses and approximately one million deaths per year have been associated with pathogenic *E. coli* [9].

Microbial foodborne illness remains a global concern. *E. coli* O157:H7 has been recognized as the most important enterohaemorrhagic *E. coli* (EHEC) serotype associated with human disease [8]. It has also emerged as the most important foodborne, zoonotic pathogen in humans [8,10]. Its importance as a public health problem was recognized in 1982, following an outbreak in the United States of America [8]. This serotype can cause hemolytic uremic syndrome (HUS), hemorrhagic colitis, abdominal cramps and diarrhea in humans [11,12]. WHO [8] estimated that more than nine percent of patients with EHEC infection may develop HUS, with a case-fatality rate of three to five percent. The intestinal tracts of cattle, sheep and goats can serve as reservoir of *E. coli* O157:H7 [13]. These animals can contaminate the environment through shedding of the organism in their feces [14,15].

The intestines of animals are considered as a major reservoir and an ideal environment for the selection and transfer of antimicrobial resistance genes. Studies have shown that *E. coli* can serve as reservoirs of antibiotic resistance genes [16] which have been efficiently transferred not only to other *E. coli* strains but also to other enteric pathogens of humans and animals [17].

Several reports on culture proven *E. coli* O157:H7 diarrheal illnesses have been documented in many African countries including Nigeria, Uganda, Gabon, Kenya, Ivory Coast, South Africa and Swaziland [13]. In 1997, in Trinidad and Tobago, a survey of wildlife revealed the presence of only one (1 out of 271, <1%), four (4 out of 175, 2%), and two (2 out of 373, <1%) *E. coli* isolates from free-ranging mammals, captive wild animals, and zoo animals, respectively, that were non-sorbitol fermenters which were not agglutinated by O157

antiserum [18]. In Grenada, a study by Sylvester et al. [19] revealed the presence of 12 (12 out of 42, 29%) *E. coli* isolates from green iguanas (*Iguana iguana*) that were non-sorbitol fermenters which were negative for agglutination using the *E. coli* O157:H7 latex kits.

There have been no published surveys on the prevalence of *E. coli* including the *E. coli* O157:H7 serotype in goats in Grenada. The objectives of the present study were to determine: the occurrence of the *E. coli* including the O157:H7 serotype in feces in a population of healthy goats and the antimicrobial drug resistance of the *E. coli* isolates.

2. MATERIALS AND METHODS

Fecal samples were obtained from 70 randomly selected healthy adult goats from 10 farms in Grenada during a three months period, May 2014 to July 2014. Each sample was immediately placed in a sterile plastic container (Starplex Scientific Corp, Cleveland, TN, USA) and 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 isolation of *E. coli*, the fecal samples were placed in 10 ml of tryptic soy broth (Remel, Lenexa, KS, USA) and incubated at 37°C for 24 hours. After incubation, an aliquot was then streaked onto MacConkey (MAC) agar (Remel, Lenexa, KS, USA) and incubated at 37°C for 24 hours. To increase the possibility of identifying *E. coli* O157:H7 in a sample, two (2) pink to red color colonies with or without a zone of precipitated bile, morphologically representing *E. coli* were subcultured via streaking onto individual MAC agar and incubated at 37°C for 24 hours for isolation of pure colonies. Colonies from the second MAC agar plate were Gram stained and further tested using the API20E (Analytical Profile Index; BioM6rieux, Hazelwood, MO) bacterial identification strips for confirmation as *E. coli*. Non-lactose fermenting isolates identified as *E. coli* by API20E were also added in the study despite the fact that they were non-lactose fermenting variants.

The identification of *E. coli* O157:H7 was performed using the methods previously described by Sylvester et al. [19]. The pure colonies were first plated on sorbitol-MacConkey agar (Remel, Lenexa, KS, USA) and incubated at 37°C for 24 hours. After incubation, the sorbitol-MacConkey agar 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 two (2) *E. coli* O157:H7 latex kits: Remel Wellcolex* *E. coli* O157 Rapid Latex Test (Remel Europe Ltd, Kent, UK) and Prolex^{TM} *E. coli* O157 Latex Kit (Pro-lab Diagnostics, Toronto, Canada). Two (2) latex kits were used to reduce the possibility of obtaining a false positive result. Any isolate giving a positive reaction to the latex test kits was considered to be *E. coli* O157:H7.

The antimicrobial susceptibility tests were carried out using the disc diffusion method as recommended by the Clinical and Laboratory Standards Institute (CLSI) using Mueller Hinton agar (BBL), and the inhibition zone sizes were interpreted as per CLSI guidelines [20]. The antibiotics discs used were ampicillin, amoxicillin-clavulanic acid, cefepime, cefotaxime, ceftazidime, ciprofloxacin, enrofloxacin, gentamicin, nalidixic acid, streptomycin, tetracycline and trimethoprim-sulfamethoxazole (Becton, Dickinson and Co., Sparks, MD, USA).

3. RESULTS

In this present study, all the 70 tested goats were culture positive for *E. coli*. A total of 140 *E. coli* isolates were recovered and analyzed for the presence of non-sorbitol fermenting colonies and agglutination. Of the 140 yielded *E. coli*, 11 (8%) isolates were non-sorbitol fermenters but only one (<1%) out of the non-sorbitol fermenters gave a positive reaction (O157-agglutinating) to the two *E. coli* O157:H7 latex kits.

Table 1 presents the details of the antimicrobial susceptibility of the 140 *E. coli* isolates recovered in this present study. The results revealed a low resistance rate (0 to 7%) for all the 140 *E. coli* isolates to five of the tested antibiotics except to streptomycin (19%). However, a number of the *E. coli* isolates revealed a moderate to high intermediate resistance rate (1 to 34%) to some of the tested antibiotics. The *E. coli* isolates recovered in this present study, were 99 to 100% susceptible to amoxicillin-clavulanic acid, ciprofloxacin, enrofloxacin, gentamicin, cefepime and ceftazidime and showed zones of inhibition measuring greater than 28 mm around each disc. Five percent (7 out of 140) *E. coli* isolates in this present study showed resistance to two or more

antibiotics. The single *E. coli* O157:H7 isolate recovered in this present study was susceptible to all the tested antibiotics.

4. DISCUSSION

The results of this study indicate that presently, healthy goats in Grenada harbor *E. coli* in their gastrointestinal tract and the occurrence is widespread among goats in the investigated farms. However, they are not major reservoirs for the *E. coli* O157:H7 serotype. Our study revealed the presence of only one (<1%) *E. coli* O157:H7 isolate. This is somewhat similar to the low rates reported in Ethiopia in 2010 where only two out of 60 (3.3%) fecal samples from goats showed the presence of *E. coli* O157:H7 serotype. The low occurrence rate of *E. coli* O157:H7 serotype observed in this present study also concurred with the low occurrence rate observed in different animals from other countries. *E. coli* O157:H7 serotype was recovered from 5.5% of sheep in Ethiopia [13], 3% of lambs in Spain [21], 0.2% of lambs in Italy [14], 4% of ewes and 4% of lambs in the Netherlands [22], and 1.4% of sheep, with monthly variations from 0% to 4.8% in the United Kingdom [15,23]. In contrast with the low occurrence rate of *E. coli* O157:H7 observed in this present study, higher occurrence rates ranging from 55% to 95% of the goats tested were reported in France [24]. In the United States of America, a prevalence rate of 43% was detected in sheep [25,26], and in Australia [27-29], occurrence rates of 40% was reported for goats, while rates ranging from of 56% to 68% were reported for sheep. In the United Kingdom, a survey of 1000 sheep at slaughter revealed an occurrence rate of 2% for *E. coli* O157:H7 [30]. On the contrary, in other reports from different parts of the world, no *E. coli* O157:H7 was found in any of the samples examined [31-34]. The difference in the number of *E. coli* O157:H7 recovered in this study and those recovered in other studies carried out in different parts of the world were probably due to the differences in husbandry practices, agro-climatic variations, sampling, methods of detection, breeds and the age of animals [14,35].

In the present study, 10 (7%) of the *E. coli* isolates were non-sorbitol fermenters that gave a negative reaction (no O157-agglutinating) to the two *E. coli* O157:H7 latex kits. This present study was designed to target only the *E. coli* O157:H7 serotype which is typically non-sorbitol fermenters that give a positive reaction to the *E. coli* O157:H7 latex kits. Hence, the 10 (7%) *E. coli* isolates that were non-sorbitol fermenters that gave a negative reaction to the two *E. coli* O157:H7 latex kits were not identified in relation to their serotypes. It is pertinent to note that some pathogenic non-O157 serotypes of *E. coli* including O26, O103, O145, O172, O174, O113 and O111, which are non-sorbitol fermenters that do not give positive reaction to *E. coli* O157:H7 latex kits exist. Some of these non-O157 groups have been previously associated with infections in humans [14,21].

Table 1. Antimicrobial susceptibility profiles of 140 *E. coli* recovered from feces of goats in Grenada between May and July, 2014

Antimicrobial (Disc conc.[a] (µg))	Resistant # (%)**	Intermediate	Susceptible
Ampicillin (10)	3 (2)	15 (11)	122 (87)
Amoxicillin-clavulanic acid (20, 10)	0 (0)	2 (1)	138 (99)
Cefepime (30)	0 (0)	0 (0)	140 (100)
Cefotaxime (30)	0 (0)	6 (4)	134 (96)
Ceftazidime (30)	0 (0)	0 (0)	140 (100)
Ciprofloxacin (5)	0 (0)	1 (1)	139 (99)
Enrofloxacin (5)	0 (0)	1 (1)	139 (99)
Gentamicin (10)	1 (1)	0 (0)	139 (99)
Nalidixic acid (30)	6 (4)	0 (0)	134 (96)
Streptomycin (10)	26 (19)	48 (34)	66 (47)
Tetracycline (30)	10 (7)	10 (7)	120 (86)
Trimethoprim-sulfamethoxazole (1.25, 23.75)	3 (2)	0 (0)	137 (98)

***#: number, % (percentage): values are rounded up and down to the nearest whole number*
[a]Resistant, intermediate or susceptible according to CLSI guideline for all drugs

In comparison with other studies, a survey carried out on wildlife in Trinidad and Tobago, revealed the presence of non-sorbitol fermenting *E. coli* that were not agglutinated by O157 antiserum from: free-ranging mammals (<1%), captive wild animals (2%) and animals in a zoo (0.5%) [18]. Sylvester et al. [19] reported a 29% (12 out of 42) prevalence rate of non-O157 serotypes of *E. coli* in green iguanas in Grenada that did not ferment sorbitol and gave negative reaction to the *E. coli* O157:H7 latex kits. This basically means that both wild and domestic animals in Grenada and other parts of the world may be harboring non-O157 group in their gastrointestinal tracts. Further studies that targets the non-O157 group is required to determine whether the 10 (7%) of the *E. coli* isolates that were non-sorbitol fermenters that gave a negative reaction to the *E. coli* O157:H7, are the pathogenic non-O157 group. This information will enable us to determine whether goats in Grenada harbor non-O157 group of *E. coli* in their gastrointestinal tracts and may possibly be a contributing source of contamination.

In this study, the result obtained from the disc diffusion test revealed low resistance rate ranging from 1% to 19% for the 140 isolates to six out of the 12 antibiotics tested (Table 1). This is somewhat similar to the findings of Sylvester et al. [19] who tested *E. coli* isolates from green iguanas to similar types of antibiotics. Their result revealed resistance rates of 7% to ampicillin, 12% to streptomycin, and 2% each to nalidixic acid, trimethoprim-sulfamethoxazole, and tetracycline. We also observed a zero resistance rate to ceftazidime, ciprofloxacin and enrofloxacin. This is also similar to the findings of Sylvester et al. [19]. In contrast, the *E. coli* isolates in this present study showed zero resistance rate to amoxicillin-clavulanic acid and cefotaxime, and 1% resistance rate to gentamicin whereas the *E. coli* isolates from the study of Sylvester et al. [19] showed a resistance rate of 12% to amoxicillin-clavulanic acid and 2% to cefotaxime, and zero resistance rate to gentamicin. On the other hand, the low resistance rate to ampicillin (2%) observed in this present study was in contrast to the high resistance rate of 84% to ampicillin reported in eastern Ethiopia [36]. Interestingly, the one *E. coli* O157:H7 isolate recovered in this present study was 100% susceptible to all the tested antibiotics. A number of the *E. coli* isolates recovered in this present study showed moderate to high intermediate resistance rates (1 to 34%) to some of the tested antibiotics and 5% (7 out of 140) of our *E. coli* isolates were resistant to two or more antibiotics. This is of public health concern since these multiple antibiotic resistant organisms can be transmitted from animals to humans.

5. CONCLUSION

Our study revealed that presently, healthy goats in Grenada are not major reservoirs for the *E. coli* O157:H7 serotype. The single *E. coli* O157:H7 isolate recovered in this present study was susceptible to all the tested antibiotics. Among the non-O157:H7 isolates, the resistance rate to drugs other than tetracycline and streptomycin was very low. Resistance to two or more antibiotics was observed only in 5% of the 140 *E. coli* isolates. This indicates that healthy goats in Grenada are presently not main reservoirs for multiple resistant *E. coli* strains.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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