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Antimicrobial Susceptibility Profiles of *Escherichia coli* Recovered from Feces of Young Healthy Domestic Pigs in Grenada, West Indies

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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 KT managed the collection of all the samples. Authors VAA, VM and EB 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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Short Research Article

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ABSTRACT

Aim: To determine the antimicrobial susceptibility profiles of *E. coli* isolates of porcine origin in Grenada, West Indies.
Study Design: During the period of May to July, 2014, rectal swab samples were collected from pigs of six weeks to 12 week of age, from randomly selected small scale pig farms from the six parishes of Grenada and analyzed in the bacteriology lab in the Pathobiology Department, School of Veterinary Medicine, St. George's University, Grenada.
Methodology: A total of 180 rectal swab samples were examined for the presence of *E. coli* by culture. All the isolates were tested against 12 antibiotics using the standard Kirby-Bauer disc diffusion method.
Results: All the 180 tested pigs were culture positive for *E. coli*. Antimicrobial susceptibility tests against 12 drugs showed susceptibility of all the *E. coli* isolates to amoxicillin-clavulanic acid, cefotaxime, ceftazidime, ciprofloxacin, imipenem, and gentamicin. Rate of resistance to tetracycline

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(96%) was the highest. Low rates of resistance to trimethoprim-sulfamethoxazole (3%), ampicillin (3%), chloramphenicol (1%), neomycin (1%) and cephalothin (1%) were observed. However, 22% of isolates showed intermediate resistance to cephalothin. Only 6% of the *E. coli* isolates showed resistance to two or more antibiotics.

Conclusion: This study showed that young healthy pigs in Grenada are not major reservoirs for multiple resistant *E. coli*. This study also confirms the inefficacy of tetracycline against *E. coli* of porcine origin.

Keywords: Rectal; culture; amoxicillin-clavulanic acid; ceftazidime; cephalothin; neomycin; MacConkey; St. David's.

1. INTRODUCTION

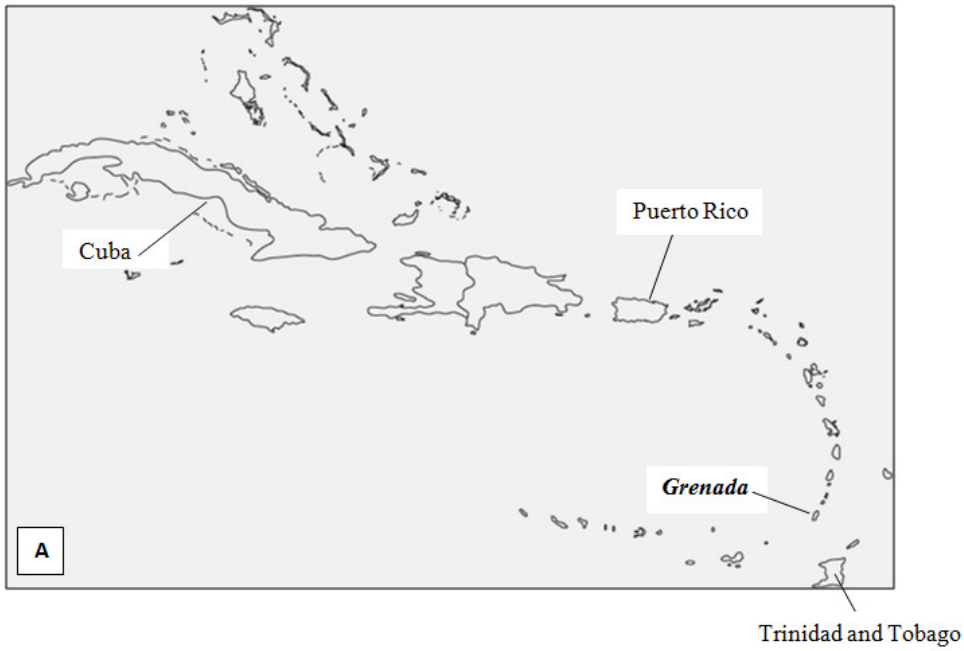
Escherichia coli is a common inhabitat of the large intestine and lower small intestine of variety of mammals [1,2]. Their numbers are usually larger in omnivores, such as pigs, and carnivores than the herbivours [2]. *E. coli* is excreted in feces and can be easily spread via soil, food and water [1,2]. A large number of *E. coli* strains are non-pathogenic [3], however, the pathogenic strains may cause severe intestinal or extra intestinal disease [4]. Studies have shown that *E. coli* can serve as reservoirs of antibiotic resistance genes [5] which have been efficiently transferred not only to other *E. coli* strains but also to other enteric pathogen of humans and animals [6]. The development of resistance in *E. coli* and other bacteria may be linked to the indiscriminate use of antibiotics in livestock systems.

Antibiotics are used in different farm animals systems to treat bacterial diseases and improve animal productivity [7]. Feed-based antibiotics usage has consistently benefit productivity of livestock, increasing the ability of farms to maintain profitable margins, allowing the consumer to purchase, at a reasonable cost, high quality meat [7,8]. However, contrasting the above benefits are suggestions that agricultural use of antibiotics may be associated with the emergence of drug-resistant organisms [9]. Antimicrobial resistance is a progressively global problem and the emerging antimicrobial resistance has become a major public health issue worldwide [10]. Although many studies have been conducted and seminars have been assembled to deal with the issue of antimicrobial resistance and emerging resistance [11], a lack of data continues to hamper efforts to develop solutions. Previous studies carried out in Grenada have shown that both wild and domesticated animal species including chicken [12-15], dogs [16], cats [17] cane toads [18] and green iguanas [19] may serve as reservoirs for antibiotic resistant bacteria and these animals can freely shed these antibiotic resistant organisms in the environment.

Analysis of antimicrobial resistance and phylogenetic groups of commensal *E. coli* isolates from healthy pigs in Grenada has been previously reported [20]. Although the resistance profile of *E. coli* recovered from healthy pigs have been monitored, continuous monitoring of the resistance profile will reveal any change in the pattern of resistance and provide information on the current susceptibility pattern of *E. coli* to drugs that are currently used for the treatment of bacterial infections in the human and veterinary clinics in Grenada. This study was designed to determine the antimicrobial susceptibility profiles of the *E. coli* isolates of porcine origin in Grenada.

2. MATERIALS AND METHODS

During the period of May to July, 2014, a total of 180 rectal swab samples were collected from pigs of six weeks to 12 week of age, from randomly selected small scale pig farms from the six parishes of Grenada: St. George's, St. Andrew's, St. John's, St. David's, St. Mark's, and St. Patrick's. A map representing the parishes where this study was conducted is presented in Fig. 1 [21]. Each parish provided 30 swab samples and all the samples were immediately stored in a cooler with ice packs and transported to the St. George's University, School of Veterinary Medicine, bacteriology laboratory and cultured within three to four hours of collection for the presence of *E. coli*. The age and sex of the pigs as well as the date and place of sample collection were recorded during the time of sample collection.



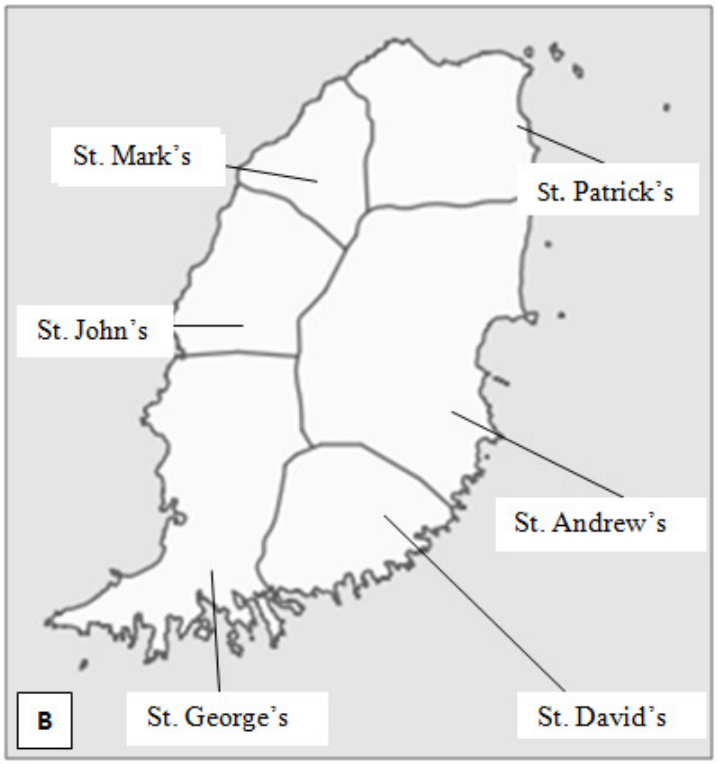


Fig. 1(A). Location of Grenada; (B) sites in Grenada where samples were collected

For the culture and identification of *E. coli*, the swabs were plated onto a MacConkey agar (MAC) (Remel, Lenexa, KS, USA) by streak plating, and incubated aerobically at 37°C for 18 – 24hrs. After incubation, one lactose or non-lactose (from plates with only non-lactose fermenting colonies) fermenting isolated colony

per sample with typical *E. coli* morphology was subcultured the second time on another MAC and incubated at 37℃ for 18 – 24hrs. 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 there were non-lactose fermenting variants.

All the isolates were tested for antimicrobial susceptibility using the standard Kirby-Bauer disc diffusion method on Mueller-Hinton agar (BBL) as recommended by the Clinical and Laboratory Standard Institute guidelines (CLSI) [22]. The antibiotics used in the study are: ampicillin, amoxicillin-clavulanic acid, cefotaxime, ceftazidime, cephalothin, chloramphenicol, ciprofloxacin, gentamicin, imipenem, neomycin, tetracycline, and trimethoprim-sulfamethoxazole (BD, Franklin Lakes, NJ). The inhibition zone sizes for all antibiotics except neomycin were interpreted based on the CLSI guidelines [22]. For neomycin, the manufacturer's guidelines, as approved by the U.S. Food and Drug Administration (FDA), were used.

3. RESULTS AND DISCUSSION

In this present study, all the 180 tested pigs were culture positive for *E. coli*. The high prevalence rate of *E. coli* (100%) observed in this study is to some extent similar to the rate reported in Grenada in 2011 where up to 90% (102 out of 113) of healthy pigs tested were culture positive for *E. coli* [1]. Previous studies have shown that *E. coli* is one of the major bacterial flora of nursing and weaned pigs [23].

The antibiotic susceptibility profiles of the 180 *E. coli* isolates recovered from the positive pigs are presented in Table 1. They revealed susceptibility of all the isolates to amoxicillin-clavulanic acid, cefotaxime, ceftazidime, ciprofloxacin, imipenem, and gentamicin. This is similar to the findings of Sabarinath, et al. [1], who reported that all the *E. coli* isolates from healthy pigs were susceptible to amoxicillin-clavulanic acid, ciprofloxacin, and gentamicin. Our *E. coli* isolates (99%) were susceptible to chloramphenicol and neomycin, and 97% were susceptible ampicillin and trimethoprim-sulfamethoxazole, whereas the study of Sabarinath, et al. [1] showed that all their *E. coli* isolates were susceptible to chloramphenicol and neomycin.

The highest rate of resistance observed in this present study was for tetracycline (96%). This concurred with the 100% resistance rate reported in Grenada in 2011 [1]. It is pertinent to note that oxytetracycline, penicillin+streptomycin, and trimethoprim-sulfadiazine are the most commonly used antibiotics for treatment of pigs in Grenada [1]. Chlortetracycline (2 grams/ton) is commonly used as an additive for pigs in Grenada [1]. This may be the reason for the high rate of resistance to tetracycline observed in this present study as well as the study in 2011 [1]. Tetracycline resistance is common in *E. coli* of porcine origin. Studies conducted in different countries on *E. coli* of porcine origin revealed a rate high or resistance to tetracycline which varied from 78% to 93% [4,24-28].

We observed a low rate of resistance to trimethoprim-sulfamethoxazole (3%), ampicillin (3%), chloramphenicol (1%), neomycin (1%) and cephalothin (1%). Twenty two percent (22%) of our *E. coli* isolates showed intermediate resistance to cephalothin. In 2011, a similar low rate of resistance to trimethoprim-sulfamethoxazole (6%) and ampicillin (3%) was observed [1]. Hariharan, et al. [4] observed a high rate of resistance to trimethoprim-sulfamethoxazole (32%) and neomycin (27%) in their study in Canada. They indicated that trimethoprim-sulfamethoxazole and neomycin are the two drugs used for treatment of porcine diarrhea due to *E. coli* in Canada. In 1989, a study on enterotoxigenic *E. coli* from swine indicated that the majority of strains resistant to trimethoprim-sulfamethoxazole tend to show resistance to tetracycline, neomycin, and ampicillin as well [6]. This concurred with the observations despite the fact that the rate of resistance to trimethoprim-sulfamethoxazole, neomycin, and ampicillin in our study was low compared to the rate of tetracycline resistance.

Six percent (10 out of 180) *E. coli* isolates in this present study showed resistance to two or more antibiotics. The rate of multiple resistance observed in our study was lower compared to the 36% and >60% rates reported in Grenada in 2011 [1] and Chile in 2005 [29], respectively, which used a similar set of antibiotics. This present study showed that there is a decrease in the rate of multiple resistance in *E. coli* of porcine origin when compared to the rate reported in 2011.

Table 1. Antimicrobial susceptibility profiles of 180 *E. coli* recovered from feces of young healthy domestic pigs in Grenada between May and July, 2014

Antimicrobial (Disc conc. [a] (µg))	Resistant	Intermediate	Susceptible
	# (%)**		
Ampicillin (10)	5 (3)	1 (1)	174 (97)
Amoxicillin-clavulanic Acid (20, 10)	1 (0)	0 (0)	180 (100)
Cefotaxime (30)	0 (0)	0 (0)	180 (100)
Ceftazidime (30)	0 (0)	0 (0)	180 (100)
Cephalothin (30)	2 (1)	39 (22)	139 (77)
Chloramphenicol (30)	1 (1)	1 (1)	178 (99)
Ciprofloxacin (5)	0 (0)	0 (0)	180 (100)
Gentamicin (10)	0 (0)	0 (0)	180 (100)
Imipenem (10)	0 (0)	0 (0)	180 (100)
Neomycin (30)[b]	1 (1)	1 (1)	178 (99)
Tetracycline (30)	173 (96)	1 (1)	4 (2)
Trimethoprim-sulfamethoxazole (1.25, 23.75)	5 (3)	0 (0)	175 (97)

***#: 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 except neomycin
[b]For neomycin, FDA-approved manufacturer's (BD) guideline were used

Based on the observation in this present study and the report of the study in 2011, the drug resistance among *E. coli* from swine in Grenada has not reached an emergency situation as previously stated in 2011 [1], however, consistent monitoring is important mainly due to the high rate of tetracycline resistance observed in this present study and in 2011 [1].

4. CONCLUSION

Our study showed that *E. coli* of porcine origin from Grenada is highly resistant to tetracycline and moderately resistant to cephalothin. The rate of resistance to trimethoprim-sulfamethoxazole, ampicillin, chloramphenicol, and neomycin was very low. Our study also revealed that presently, young healthy pigs in Grenada are not major reservoirs for multiple resistant *E. coli*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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