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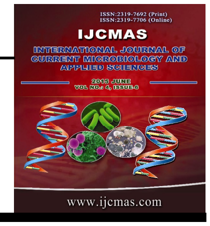

Original Research Article

Antimicrobial Resistance Profiles of *Escherichia coli* Recovered from Feces of Healthy Free-Range Chickens in Grenada, West Indies

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A B S T R A C T

Keywords

Escherichia coli, Antimicrobial resistance, Gentamicin, Amoxicillin-clavulanic acid, Cephalothin, Free-range, Chickens, Grenada, West Indies

Free-range chickens are kept by most of the households in Grenada, as a source of food and limited income generation. Antimicrobial resistance is among the major threat to global public health and it is increasing in high rates in developing countries. In this study, we determined the occurrence of *Escherichia coli* in feces of healthy free-range chickens in Grenada, by culture and their antimicrobial resistance by Kirby-Bauer disc diffusion method. A total of 202 *E. coli* isolates from cloacal swabs were tested for susceptibility to antimicrobial drugs of veterinary and human importance. Of the 202 *E. coli* isolates, 35.1% (71 of 202) showed resistance to at least one out of the 12 antibiotics tested whereas 31% (22 of 71) showed resistance to two or more antibiotics. The most common resistance observed was against tetracycline (29.7%), followed by ampicillin (6.9%), gentamicin (5.9%), and trimethoprim-sulfamethoxazole (3%) while the least common resistance observed was against amoxicillin-clavulanic acid (0.5%), cephalothin (1%), and ciprofloxacin (1%). Since the sampled chickens do not receive any medication, it is evident that the environment is the plausible source of resistant bacteria. There is a risk of resistant bacteria being transmitted to humans via contaminated poultry products and the environment.

Introduction

Free-range chickens are an essential resource benefiting the livelihoods of poor people in developing countries (Msoffe *et al.*, 2010) including Grenada which is a small volcanic island in the southeastern Caribbean with approximately 344 km^2 land size (Central Intelligence Agency, 2013).

These chickens serve as an important source of food and limited income generation for the rural poor (Msoffe *et al.*, 2010). In 2012, it was estimated that the population of free-range chickens in Grenada was approximately 25, 688 birds with the number of birds per household ranging between 5 and 40 (Dr. Louison, Chief Veterinary Officer, Ministry of Agriculture, Lands, Forestry, Fisheries & Environment, Grenada: Personal communication, February, 2013). The flocks comprises of cocks, hens, and chicks. Many of the households in Grenada keep free-range chickens given that they only require minimal resources and investments to maintain since they are scavengers almost taking care of themselves. Shelters are only provided for the chickens during the night and they are set free during the day. These chickens are usually managed under the extensive system, and are neither vaccinated nor given any antibiotic medication. They mainly feed on food remains, grasses and other waste which are dumped in the environment which expose them to getting in contact with pathogenic bacteria as well as antimicrobial resistant bacteria in the environment. Hamisi *et al.* (2014) indicated that in the developing countries, chickens are among the domestic animals threatened by being exposed to bacteria carrying resistance genes in the environment where they feed.

It has been reported that *E. coli* is amongst the common pathogenic bacteria that affects chicken production (Raji *et al.*, 2003; Wang *et al.*, 2013). *E. coli* is ubiquitous and commonly found in the gastrointestinal tract of healthy warm-blooded mammals (Markey *et al.*, 2013; WHO, 2011) including chickens. It is shed in the feces and can easily spread to other animals and humans via soil, food and water (Markey *et al.*, 2013; WHO, 2011). Pathogenic *E. coli* strains have been associated with severe intestinal or extra intestinal disease in humans (Santos *et al.*, 2013), and are considered zoonotic (WHO, 2011). In birds, pathogenic *E. coli* strains have been associated with a range of diseases including septicemia, enteritis, coligranuloma, omphalitis, arthritis, salpingitis, and complicated air sacculitis (Cheville and Arp, 1978; Ozawa *et al.*, 2008). Their role in chronic respiratory diseases in meat chicken breeds has been documented (McClenaghan *et al.*, 1981).

Antimicrobial drug resistance is becoming a major threat to global public health. It is not only a threat in the treatment of poultry diseases associated with *E. coli*, but also a potential public health hazard to individuals that consume poultry products (Hariharan *et al.*, 2008). Several accessible reports have shown that antibiotic drug resistance is increasing among commensal and pathogenic bacterial isolates from both humans and animals (Linton *et al.*, 1988; Salyers *et al.*, 2004). Thus, it is pertinent to investigate the emerging resistance patterns in order to determine control strategies (Levy, 2002; Okeke *et al.*, 2005). Hamisi *et al.* (2014) mentioned that the extensive use of antibiotics in livestock is among the factors that can lead to the spread of antimicrobial resistance. Indiscriminate use of antibiotics also play an important role in the dissemination of antimicrobial resistance (Gootz, 2010; Hart and Kariuki, 1998; Nweneka *et al.*, 2009). Though many research and seminars have been conducted to manage the issue of antimicrobial resistance and emerging resistance (Gustafson, 1991), a lack of data continues to impede efforts to develop solutions. Previous studies in Grenada have shown that both wild and domesticated animal species including chicken (Arathy *et al.*, 2011; Hariharan *et al.*, 2008; Hariharan *et al.*, 2007), pigs (Amadi *et al.*, 2015; Sabarinath *et al.*, 2011), cats (Hariharan *et al.*, 2011), and green iguanas (Sylvester *et al.*, 2014) serve as reservoirs for antimicrobial resistant *E.coli*. As these animals harbor resistant *E. coli* in their intestines, the animals can easily shed the resistant organisms into the environment.

There have been no published surveys on the antimicrobial resistance of *E. coli* recovered from free-range chicken in Grenada. The purpose of this present study was to determine the occurrence of *E. coli* in feces of healthy free-range chickens and their antimicrobial resistance in Grenada.

Material and Methods

Sample collection: From May to July, 2014, a total of 205 fecal samples were collected by cloacal swabs from apparently healthy backyard chickens from six parishes of Grenada: St. Patrick's (n = 48), St. David's (n = 38), St. George's (n = 37), St. Andrew's (n = 35), St. John's (n = 35), and St. Mark's (n = 12). After collection, the cloacal swabs were immediately stored in a cooler with ice packs and transported to the St. George's University, School of Veterinary Medicine, Bacteriology laboratory where all the laboratory analysis where performed. The approximate time between sample collection and analysis was three hours.

Isolation and identification of E. coli: Each sample was inoculated 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°C 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 due to the fact that there were non-lactose fermenting variants.

Antimicrobial susceptibility analysis: All the identified isolates were tested for susceptibility to ampicillin (10µg), amoxicillin-clavulanic acid (30µg), cefotaxime (30µg), ceftazidime (30µg), cephalothin (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), gentamicin (10µg), imipenem (10µg), neomycin (30µg), tetracycline (30µg), and trimethoprim-sulfamethoxazole (25µg) (BD, Franklin Lakes, NJ) by determining the inhibitory zone sizes using the standard Kirby-Bauer disc diffusion method on Mueller-Hinton agar (BBL) as recommended by the Clinical and Laboratory Standard Institute guidelines (CLSI) (CLSI, 2008). The inhibition zone sizes for all antibiotics except neomycin were interpreted as per CLSI guidelines (CLSI, 2008). For neomycin, the manufacturer's guideline, as approved by the U.S. Food and Drug Administration (FDA), was used.

Results and Discussion

In this study, a total of 205 chickens were examined of which 202 (98.5%) were culture positive for *E. coli*. Thus, a total of 202 *E. coli* isolates were recovered (one *E. coli* isolate per positive chicken). Of the 202 *E. coli* isolates, 35.1% (71 of 202) showed resistance to at least one out of the 12 antibiotics tested while 31% (22 of 71) showed resistance to two or more antibiotics. Overall, our study revealed a low resistance rate of all the 202 *E. coli* isolates to seven out of the 12 antibiotics tested (Table 1). The most common resistance observed was against tetracycline (29.7%), followed by ampicillin (6.9%), gentamicin (5.9%), and trimethoprim-sulfamethoxazole (3%) while the least common resistance observed was against amoxicillin-clavulanic acid (0.5%), cephalothin (1%), and ciprofloxacin (1%).

This is the first study in Grenada to investigate antimicrobial resistance in *E. coli* isolates from tropical free-range Chickens. In the present study, *E. coli* strains were detected in 98.5% (202 of 205) of the fecal samples. This is not surprising since it is consistent with the prior knowledge that *E. coli* is a normal gut flora in endotherms which is frequently shed in feces. The fecal samples (1.5%, 3 of 205) from which *E. coli* strain was not detected may have been collected from the chickens that were not actively shedding the bacteria at the time of our sample collection. Gordon and Cowling (2003) indicated that environmental condition, geographical location and/or other host factors can affect the distribution of *E. coli* among animals of the same species. This could also be a possible reason why the chickens (1.5%) where not actively shedding the bacteria.

Our antimicrobial susceptibility assay revealed that 35.1% (71 of 202) of the *E. coli* isolates were resistant to at least one of the tested antibiotics while 31% (22 of 71) showed resistance to two or more antibiotics. Our observation did not correspond with the findings of Hamisi *et al.* (2014) who analyzed *E. coli* isolates from tropical free-range chickens from Tanzania. In their study, 83.1% (64 of 77) of the *E. coli* isolates showed resistance to at least one antimicrobial agent, while 84.4% (54 of 64) were resistant to two or more antibiotics. Another study which was carried out on wild birds and free-range poultry from Bangladesh showed a higher number of *E. coli* isolates (35 out of 66, 53%) that showed resistance to at least one of the tested antimicrobial compound (Hasan *et al.,* 2012). In 2010, a study carried out on broiler chickens in northern and central Uganda by Majalija *et al.* (2010) also revealed a higher number of *E. coli* isolates (168 out of 182, 92.3%) that were resistant to at least a single antimicrobial tested.

The resistance rates to tetracycline (29.7%), ampicillin (6.9%), and trimethoprim-sulfamethoxazole (3%) observed in our study is contrary to the results of other researchers who tested *E. coli* strains isolated from chickens against similar antibiotics: the study of Hamisi *et al.* (2014) revealed a higher resistance rates to tetracycline (75.3%), ampicillin (63.6%), and trimethoprim-sulfamethoxazole (53.3%).

In 2012, a study carried out on free-range chickens in Abeokuta, Nigeria also showed a higher resistance rates to tetracycline (76.9%) and ampicillin (92.3%) (Ojo *et al.,* 2012). Furthermore, the *E. coli* isolates in our study showed zero resistance rate to chloramphenicol, cefotaxime, and neomycin as well as a low resistance rate to ciprofloxacin (1%) which is in contrast to the resistance rate to chloramphenicol (5.2%) and cefotaxime (29.9%) observed by Hamisi *et al.* (2014). Our observation is also contrary to the high resistance rate to chloramphenicol (73.1%), ciprofloxacin (34.6%), and neomycin (76.9%) reported by Ojo *et al.* (2012).

The rate of *E. coli* resistance to tetracycline (29.7%), ampicillin (6.9%), trimethoprim-sulfamethoxazole (3%), and the zero resistance to chloramphenicol observed in our study is lower than that observed in *E. coli* isolates from broilers chickens in central and northern Uganda (tetracycline 31% and 55%, ampicillin 44% and 87.2%, trimethoprim-sulfamethoxazole 17% and 41.3%, and chloramphenicol 8% and 13.8% from central and northern Uganda, respectively (Majalija *et al.,* 2010). The resistance rate to ciprofloxacin (1%) observed in our study is somewhat similar to the resistance rate to ciprofloxacin 0% and 2.8% observed in the central and northern Uganda, respectively (Majalija *et al.,* 2010).

Table.1 Antimicrobial susceptibility profiles of 202 *E. coli* recovered from feces of healthy free-range chickens in Grenada between May and July, 2014

Antimicrobial (Disc conc. [a] **(µg))**	**Resistant**	**Intermediate**	**Susceptible**
	# (%)**		
Ampicillin (10)	14 (6.9)	2 (1)	186 (92.1)
Amoxicillin-clavulanic Acid (20, 10)	1 (0.5)	2 (1)	199 (98.5)
Cefotaxime (30)	0	1 (0.5)	201 (99.5)
Ceftazidime (30)	0	0	202 (100)
Cephalothin (30)	2 (1)	41 (20.3)	159 (78.7)
Chloramphenicol (30)	0	0	202 (100)
Ciprofloxacin (5)	2 (1)	0	200 (99)
Gentamicin (10)	12 (5.9)	0	190 (94.1)
Imipenem (10)	0	0	202 (100)
Neomycin (30)[b]	0	2 (1)	200 (99)
Tetracycline (30)	60 (29.7)	10 (5)	132 (65.3)
Trimethoprim-sulfamethoxazole (1.25, 23.75)	6 (3)	1 (0.5)	195 (96.5)

**#: 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.

In 2008, a study on commercial chickens (broilers and layers) from Grenada by Hariharan *et al.* (2008) showed a higher resistance rate to tetracycline (58.5%; broilers 66.7% and layers 37.3%) in comparison with the resistance rate to tetracycline (29.7%) observed in our study, However, the resistance rate to chloramphenicol (0.55%) somewhat correspond with the zero resistance rate to chloramphenicol observed in our study.

A few number of our *E. coli* isolates showed resistance to fluoroquinolone drug, ciprofloxacin (1%, 2 of 202). This concurred with the findings of Hariharan *et al.* (2007), who also reported a low resistance rate to ciprofloxacin (2%, 4 of 202).

One plausible explanation for the high resistance rate to tetracycline is observed in our study and other studies including those carried out in commercial poultry is the excessive use of tetracycline which is the common therapeutic antibiotics used in poultry production (Fairchild *et al.*, 2005). Although free-range chickens do not normally receive antibiotics for the prevention and treatment of bacterial infections or for growth promotion purposes (Okoli, 2006), it is possible that the *E. coli* in our studies acquired resistance to tetracycline through transmissible plasmids from humans and other animals as well as the environment due to the close contact and proximity of the habitats of the free-range chicken to human settlements and livestock farms. Tetracycline-resistant bacteria have been recovered from humans, animals, and the environment, with the majority of *tet* genes associated with mobile plasmids or transposons (Chopra and Roberts, 2001).

Intermediate resistance in our study was most common against cephalothin (20.3%) and tetracycline (5%), but the least common against ampicillin (1%), amoxicillin-clavulanic acid (1%), cefotaxime (0.5%), neomycin (1%), and trimethoprim-sulfamethoxazole (0.5%). Continuous monitoring of antimicrobial resistance pattern in chicken especially the free-range chickens is necessary in Grenada in order to monitor determine the development and level of bacterial resistance to drugs that are used for the treatment of bacterial infection in humans such as the cefotaxime which is the first line drug used for the treatment of severe enteric bacterial infections in human medicine (Threlfall *et al.,* 2000).

In conclusion, this study revealed that presently, healthy free-range chickens in Grenada are reservoirs for the *E. coli*. However, the resistance rate to antibiotics seen among *E. coli* isolates is low, compared to studies in other tropical countries probably because the free-range chickens in Grenada do not normally receive medications for therapeutic or growth promotion purposes. Notably, occurrence of multidrug resistant *E. coli* exhibiting resistance to two or more antibiotics poses threat to the public health despite the fact that the rate was moderately high. It is important to continue monitoring antibiotic resistance pattern in bacteria isolated from free-range chickens in developing countries such as Grenada so as to elucidate the role played by the chickens in the maintenance and dissemination of antimicrobial resistance determinants in animals and humans.

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