Antimicrobial Drug Resistance in *Escherichia coli* Isolated from Commercial Chicken Eggs in Grenada, West Indies

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ABSTRACT

Objective: *To determine the antimicrobial resistance profile of Escherichia coli* (E coli) *isolated from the shell membrane and yolk of commercial chicken eggs in Grenada.*

Methods: *A total of 450 eggs were collected from different locations including small (33.3%) and big farms (26.7%), roadside vendors (26.7%) and supermarkets (13.3%). The shell membranes and yolk were cultured separately on blood agar and McConkey Agar. Escherichia coli were identified using biochemical tests and API20E strips. The isolates were tested for antimicrobial sensitivity.*

Results: *A total of 55* E coli *isolates were obtained. Of which 34 isolates were from shell membrane and 21 from yolk samples. Twenty-two of the total 34 isolates from shell membrane exhibited resistance to one or more of the antibiotics used in the study whereas 11 of the 21 yolks isolate also showed resistance to one or more of the tested antibiotics.*

Among the six antibiotics tested, the highest level of resistance was observed for ampicillin, 42.9 per cent and 31.8 per cent respectively for shell membrane and yolk isolates. The lowest resistance rate among all the antibiotics was observed against enrofloxacin (0%). Multi-drug resistance (resistance to ≥ 3 compounds) was observed in 10.9% of the isolates.

Conclusions: *This study on* E coli *drug resistance in commercial chicken eggs in Grenada generated baseline data indicating that chicken eggs used for food can harbour resistant* E coli. *A regular monitoring of commensal and clinical isolates of* E coli *for antibacterial resistance is warranted.*

Keywords: Antibiotic resistance, eggs, escherichia coli, Grenada

Resistencia Antimicrobiana a los Medicamentos en *Escherichia coli* Aislada de Huevos Comerciales de Gallina en Granada, West Indies

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RESUMEN

Objetivo: *Determinar el perfil de resistencia antimicrobiana de* Escherichia coli (E coli) *aislada de la membrana de la cáscara y la yema de huevos comerciales de gallinas en Granada.*

Método: *Un total de 450 huevos fueron recolectados de diferentes localidades, incluyendo granjas pequeñas (33.3%) y grandes (26.7%), vendedores a la orilla de los caminos (26.7%), y los supermercados (13.3%). Las membranas de la cáscara y yema fueron puestas por separado en un cultivo de agar sangre y agar McConkey. Las Escherichia coli fueron identificadas usando pruebas bioquímicas y tiras API20E. Los aislados fueron sometidos a pruebas para determinar su sensibilidad antimicrobiana.*

Resultados: *Se obtuvo un total de 55 aislados de E coli. De estos, 34 aislados provenían de la membrana de las cáscaras y 21 de las muestras de yemas. Veintidós del total 34 aislados de la membrana, mostraron ser resistentes a uno o más de los antibióticos usados en el estudio, mientras que 11 de los 21 aislados de yemas también mostraron resistencia a uno o más de los antibióticos probados. Entre los seis antibióticos probados, el nivel más alto de resistencia se observó frente a la ampicilina, 42.9 por ciento y 31.8 por ciento respectivamente para la membrana de la cáscara y los aislados de*

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yema. La tasa de resistencia más baja entre todos los antibióticos se observó frente a la enrofloxacina (0%). Una resistencia multidroga (resistencia a ≥ 3 compuestos) se observó en 10.9% de los aislados. ***Conclusiones:*** *Este estudio sobre la resistencia medicamentosa de E en los huevos comerciales de gallina en Granada, generó datos básicos que indican que los huevos de gallina usados para la alimentación pueden ser fuente de E coli resistentes a los medicamentos. Se garantiza un monitoreo regular de aislados comensales y clínicos de E coli a fin de detectar su resistencia antibacteriana.*

Palabras claves: Resistencia antibiótica, huevos, *Escherichia coli,* Granada

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INTRODUCTION

Antibiotics are used by the poultry industry and poultry veterinarians to enhance growth and feed efficiency, and to reduce disease. Antibiotic usage has facilitated the efficient production of poultry, allowing the consumer to purchase, at a reasonable cost, high quality meat and eggs (6). However, in recent years, antimicrobial resistance is an increasingly global problem and emerging antimicrobial resistance has become a public health issue worldwide (10). A variety of foods and environmental sources harbour bacteria that are resistant to one or more antimicrobial drugs used in human or veterinary medicine and in food-animal production (1, 2).

Escherichia coli is a common inhabitant of the intestinal tract of mammals and it can be easily spread through water, soil and food. Most strains are harmless (9) but others are capable of causing either intestinal or extra-intestinal diseases (15). Because of the indiscriminate use of antibiotics in poultry, *E coli* has developed resistance to some of these agents that has resulted in failures in the treatment of the infectious diseases caused by *E coli*. Moreover, *E coli* can serve as reservoirs of resistance genes (19) which have been efficiently exchanged not only with each other but also with other enteric pathogens of humans and animals (14).

Analysis of antimicrobial drug resistance and genetic diversity of commensal *E coli* isolated from caeca of broiler and layer chickens in Grenada have already been reported (8). Though many bacteria recovered from poultry or poultry-related samples have been monitored, few published studies have reported on antimicrobial resistance in *E coli* recovered from commercial chicken eggs used for food and many food products (3, 4, 12). This paper reports the antimicrobial resistance profiling of *Escherichia coli* isolated from the shell membrane and yolk of commercial chicken eggs in Grenada.

MATERIALS AND METHODS

A total of 450 eggs were collected from different locations such as small (33.3%) and big farms (26.7%), roadside vendors (26.7%) and supermarkets (13.3%, only locally produced eggs). The eggs were transported to the laboratory and cultured within six-hours of collection. The outer surface of the eggs was disinfected by wiping with surgical gauze soaked in 5% iodine solution and opened around the air sac area. After draining the albumin, the yolk with intact vitelline membrane was transferred to a sterile beaker. The shell membranes were peeled off the shell and collected aseptically. Yolks from five samples were pooled to form one sample; similarly the shell membranes from the same five eggs formed a sample.

The pooled yolk samples were incubated at 37°C for 24-hours and then 1 ml of the yolk inoculum was cultured on blood agar and McConkey agar plates and incubated for 24-hours at 37°C (7). The shell membranes were ground with trypticase soy broth using a sterile mortar and pestle and were incubated at 37°C for 24-hours and then cultured on blood agar and McConkey Agar. Plates that did not show any growth were incubated for a further 24-hours. The bacterial colonies having the appearance of *E coli* were picked up and were tentatively identified using procedures described by Cowan, 1997 (5), and confirmed as *E coli* using API20E strips (BioMerieux, Mary-IEtoile, France).

The isolates were tested for antimicrobial sensitivity using Kirby-Bauer method on Mueller-Hinton agar. All 55 isolates were subjected to antimicrobial sensitivity tests and results were interpreted as per NCCLS guidelines (13). Antibiotics used in the study are: ampicillin (AM) 10 μg, amoxicillin-clavulanic (AMC) acid 30 μg, gentamicin (GM) 10 μg, sulfamethoxasole-trimethoprim (SXT) 25 μg, enrofloxacin (ENO) 30 μg and tetracycline 30 μg. Multidrug resistance was defined as resistance to three or more classes of drugs.

RESULTS

From 180 samples (90 yolks and 90 shell membrane), a total of 55 *E coli* isolates were obtained. Of which 34 isolates were from shell membranes and 21 from yolk samples. Twenty-two of the total 34 isolates from shell membranes exhibited resistance to one or more of the antibiotics used in the study whereas 11 of the 21 yolk isolates also showed resistance to one or more of the tested antibiotics.

The resistance rates of *E coli* isolates to individual antibiotics are shown in Fig. 1 and the phenotype pattern is given in Table 1. The resistance patterns were similar for isolates from shell membrane and yolk samples. Among the six antibiotics tested, the highest level of resistance was observed for ampicillin, 42.9 per cent and 31.8 per cent respectively by shell membrane and yolk isolates. Following ampicillin, the next maximum resistance was against tetracycline. The resistance rate for any of the tested antibiotics was higher

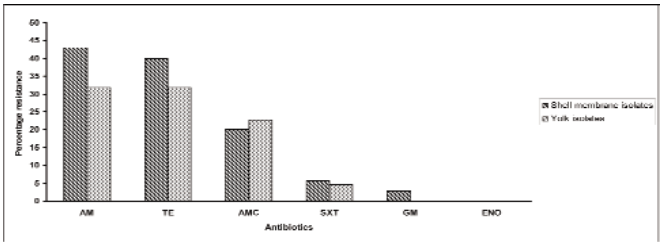

Fig.1: Resistance rate of *E Coli* isolates from shell membrane and yolk of table eggs

Table 1: Phenotype of antibiotic resistance detected among the 33 isolates of *E coli* recovered from commercial chicken eggs

Phenotypes of antibiotic resistance	Number of isolates	
	Shell membrane	Yolk
AM	3	3
TE	6	2
AM+AMC	5	4
AM+TE	4	–
AM +TE+ SXT	1	–
AM+AMC+TE+SXT	2	1
AM+AMC+TE+GM	1	–
AM+AMC+TE+SXT+GM	–	1
Total	**22**	**11**

for shell membrane isolates than yolk isolates. The lowest resistance rate among all the antibiotics was observed against enrofloxacin (0%).

Multi-drug resistance (resistance to ≥ 3 compounds) was observed in only 10.9% of the isolates (4 shell membrane isolates and 2 yolk isolates). The most common resistance pattern observed was ampicillin and amoxicillin and the entire multi-drug pattern included simultaneous resistance to ampicillin and amoxicillin.

DISCUSSION

Ever since their discovery in the 1940s, the antibiotics have been widely used for both human and veterinary medical practice (17). However, many pathogens have developed resistance to antibiotics. Resistant bacteria are routinely isolated from poultry specimens. However, little attention has been given to antibiotic resistance of bacteria isolated from commercial chicken eggs.

Resistance patterns exhibited by the *E coli* isolates in the present study from commercial chicken eggs are similar to fecal isolates from seagulls in Portugal and fecal isolates from food producing animals in many European countries (18, 16). In the previous report on *E coli* isolates from caeca of broiler and layer chickens in Grenada, the highest level of resistance was shown against tetracycline (58.5%) and the resistance against ampicillin was only 20% (7), whereas we found more resistant *E coli* strains for ampicillin. Many *E coli* isolates from poultry have demonstrated resistance against tetracycline (11, 12). Sulphonamides and tetracyclines are some of the oldest drugs used in infectious diseases, and it is not surprising that some resistance for these antimicrobials would have developed over time (17). Resistance to the β-lactam group of antibiotics was surprising since these drugs are not routinely used for treatment in commercial layers in Grenada. This indicates the necessity of proper handling of eggs to ensure the safety of consumers. Resistance to gentamicin was observed only in shell membrane isolates and is always associated with resistance to other drugs. This is similar to the gentamicin resistance reported in the caecal *E coli* isolates from broilers and layers in Grenada (8).

The present study on *E coli* drug resistance in commercial chicken eggs in Grenada generated baseline data indicating that chicken eggs used for food can harbour resistant *E coli*. Regular monitoring of commensal and clinical isolates of *E coli* for antibacterial resistance is warranted.

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