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Frequency of antimicrobial resistance of *E. coli* isolates from dairy farms in Trinidad by source and presence of virulence markers

Rohini Roopnarine, David Ammons, and Abiodun Adewale Adesiyun*

School of Veterinary Medicine, Faculty of Medical Sciences, University of the West Indies, St. Augustine, Trinidad and Tobago

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ABSTRACT

A cross-sectional study was conducted on 50 dairy farms in Trinidad. Faecal samples were collected from cows, calves and humans; rectal swabs from pet dogs as well as bulk milk and milking parlour effluent. The disc diffusion method was used to detect resistance to eight antimicrobial agents amongst 500 isolates of *Escherichia coli*. The vero cell assay was used for verocytotoxin (VT) and heat-labile (LT) detection while the polymerase chain reaction (PCR) was used to detect VT genes amongst selected isolates. In Waller Field, 70.0% (168 of 240) of the *E. coli* isolates exhibited resistance to one or more antimicrobial agents compared with 83.5% (217 of 260) of the isolates from Carlsen Field ($P < 0.001$; χ^2). Overall, the frequency of resistance of isolates was highest to streptomycin (72.7%) and lowest to the fluoroquinolones (0.2%). Of a total of 130 *E. coli* isolates positive for O157 strain, VT gene, VT and or LT production, 104 (80.0%) were resistant. Resistance amongst *E. coli* strains with virulence markers could pose therapeutic problems on dairy farms in Trinidad.

Key words: antibiograms, *E. coli*, dairy farms, Trinidad

Introduction

In the livestock industry, antimicrobial agents are used in chemoprophylaxis, chemotherapy and as growth promoters (ADESIYUN and KAMINJOLO, 1992; ORDEN et al., 1999; HEUVELINK et al., 1998). It has been documented in the literature that misuse or abuse of the agents has the potential to result in an increase in resistance amongst bacteria (DUFFY et al., 2006; ORRETT and SHURLAND, 1998; WALSH et al., 2006; WHITE et al., 2001). Resistance amongst *E. coli* strains in dairy cattle and their milk have been reported to range from 55% to 69.6% in several studies (MORA et al., 2005; YOU et al., 2006; ADESIYUN et al., 1997b).

*Corresponding author:

Prof. A.A. Adesiyun, Faculty of Medical Sciences, School of Veterinary Medicine, University of the West Indies, St Augustine, Trinidad and Tobago, West Indies, Phone: +1 868 645 4481; Fax: +1 868 645 7428; E-mail: aadesiyun@sta.uwi.edu

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Resistance to antimicrobial agents acts as an epidemiological marker for verocytotoxigenic (VTEC) strains (IZUMIKAWA et al., 1996) and is well recognised as a problem of worldwide importance (COHEN, 2000). Antimicrobial agents are believed to induce verotoxin release by bacteria and thus are avoided in treatment of humans infected with VTEC strains (MORA et al., 2005). Despite this, evidence is growing that antimicrobial resistance in humans is related to use of antimicrobial agents in food-producing animals, which has led to the development of selection pressures favouring resistant forms of bacteria (Da COSTA et al., 2008). Also, resistance genes are known to be transferred between bacteria and across species leading to multi-drug resistance (SCHJORRING et al., 2008). This could have therapeutic implications in both human and veterinary medicine.

In Trinidad and Tobago, reports on the resistance to antimicrobial agents exist for *E. coli* strains isolated from pet dogs (ADESIYUN et al., 1997a), diarrhoeic and non-diarrhoeic livestock (ADESIYUN and KAMINJOLO, 1992), captive and free-ranging wildlife (ADESIYUN and DOWNES, 1999; GOPEE et al., 2000) and from dairy cows and raw milk (ADESIYUN et al., 1997b). To date however, there is dearth of information on the antimicrobial resistance of *E. coli* strains with virulence genes or VT-producers. Also, there is no published report on the antibiograms of *E. coli* strains isolated from dairy farm workers. The study was therefore conducted to determine the frequency of resistance to commonly used antimicrobial agents amongst *E. coli* strains isolated from dairy cattle, raw milk, and farm environments. The investigation also assessed resistance amongst *E. coli* isolates from farm pet dogs as well from dairy farmers and members of their families.

Materials and methods

Duration of sampling. Samples were taken during the period January-April 2004.

Source of samples. On each farm, wherever the animal population (livestock, pets) or number of humans in the household was less than five, all individuals were sampled. On larger farms the strategy was to sample 10% of the population.

Selection of farms. The study design was to conduct the study in the two major dairy farming areas in Trinidad, Carlsen Field and Waller Field. Initially, all dairy farmers in the two areas were approached to solicit their participation in the study and to respond to a questionnaire. Twenty-five farmers were selected from each farming area using a systematic random sampling approach amongst farmers who agreed to participate.

Sample collection. The procedure described by ROOPNARINE et al. (2005) was used in the study. Briefly, on each farm, wherever the animal population (livestock, pets) or number of humans in the household was less than five, all individuals were sampled. On larger farms the strategy was to sample 10% of the population. Freshly voided faeces were collected into sterile plastic faecal cups, rectal swabs were obtained and inserted into

Amies transport medium (Oxoid Ltd., Basingstoke, Hampshire, U.K) and transported to the laboratory ice-cooled and processed within 4 hours of collection. For human samples, faecal cups were left with the individual(s) and collected the following day with requests that they be refrigerated overnight. For milk samples, approximately 25 mL were pooled from all churns of the morning milking and put into sterile universal bottles and taken to the laboratory ice-cooled within one hour of collection. Water samples, from effluents in running drains of dairy farms were collected in sterile universal bottles and transported to the laboratory ice-cooled within 1 hour of collection.

Administration of questionnaire. A standardized pre-tested questionnaire was administered to all dairy farmers in both Waller Field and Carlsen Field. The questionnaire attempted to elicit information such as number of dairy cattle (calves and cows, pet animals (dogs and cats), human beings; use of state and private veterinarians and degree of closeness between pet animals and their human owners. The principal author administered the questionnaires to all dairy farmers. To determine the degree of closeness with their dogs and cats, they were asked if the dogs were strictly guard, guard and pet or strictly pet animals and whether they handled their dogs and cats closely. Based on their responses they were classified as very close, moderately close and no close contact.

Isolation and identification of Escherichia coli. The procedure described by ROOPNARINE et al. (2005) was used.

Source of isolates of E. coli. Of a total of 933 *E. coli* isolates obtained from the 50 dairy farms regardless of source, the study design was to test 500 randomly selected isolates. Of these, 260 were from Carlsen Field and 240 from Waller Field. The isolates were randomly selected from all strains of *E. coli* recovered from the various sources and farms based on proportional representation.

Assay for verocytotoxin (VT) and heat-labile toxin (LT) production. The procedure described by ROOPNARINE (2005) was used.

Detection of VT genes. Polymerase chain reaction (PCR) was used to assay for VT1, VT2, *eae* and O157 *rfb*E genes as previously described (ROOPNARINE, 2005).

Selection of antimicrobial agents. The antimicrobial agents selected were based on antimicrobial agents used regularly by State veterinarians and farmers in Trinidad and Tobago. These included ampicillin, streptomycin, oxytetracycline and sulphamethoxazole /trimethoprim. In Waller Field, it was reported that enrofloxacin was occasionally used by veterinarians. The remaining antimicrobial agents used in the study were based on published reports in Trinidad and Tobago (ADESIYUN and KAMINJOLO, 1992) and elsewhere (SCHROEDER et al., 2002).

Determination of the antibiograms of E. coli isolates. The antibiograms of *E. coli* isolates were determined using the Kirby-Bauer disc diffusion method on Mueller-

Hinton agar (ADESIYUN and KAMINJOLO, 1992). The following antimicrobial agents on discs (Oxoid Ltd., Basingstoke, and Hampshire, U.K.) and concentrations used in the study were: ciprofloxacin (CIP, 5 μg), gentamycin (CN, 10 μg), streptomycin (S, 10 μg), oxytetracycline (OT, 30 μg), sulphamethoxazole/ trimethoprim (SXT, 23.25 μg /1.75 μg), cephalothin (CF, 30 μg), enrofloxacin (ENRO, 5 μg) and ampicillin (AMP, 10 μg). The *E. coli* ATCC strain 25922 was used as a control.

The sensitivity of each *E. coli* isolate to the antimicrobial agents was interpreted according to the guidelines set by the Clinical Laboratory Standards Institute (CLSI) (ANONYM., 2002) chart for zones of susceptibility and resistance for each antimicrobial agent used. For enfloxacin, since no human *E. coli* standards are available those for animals were used for isolates from all sources.

Statistical analysis. The frequencies of resistance to antimicrobial agents amongst *E. coli* isolates were analysed using alpha at 0.05 with Epi-info (version 6.04, Centers for Disease Control and Prevention, U.S.A) following the processing of the data using the Statistical Package for Social Sciences (SPSS) version 9.0. The Chi square (χ^2) test was used to determine the statistically significant differences between locations for antimicrobial data.

Results

The prevalence of antimicrobial resistance amongst *E. coli* isolates from both areas is shown in Table 1. In Waller Field, 70% (168 of 240) and Carlsen Field, 83.5% (217 of 260) of the isolates were resistant to 1 or more antimicrobial agents, the difference being statistically significant ($P<0.05$; χ^2).

The frequency of resistance to the eight antimicrobial agents was statistically significantly different ($P<0.05$; χ^2) as was the frequency of resistance to streptomycin between the two areas. Similarly, for cephalothin, 17.5% of isolates from Waller Field and 30.0% from Carlsen Field were resistant and the difference was statistically significant ($P<0.05$; χ^2).

The prevalence of resistance to antimicrobial agents amongst isolates according to sample source is shown in Table 2. The highest frequency of resistance to streptomycin was noted in isolates from cats (100%) followed by bulk milk (92.9%) with the lowest found for isolates from calves (69.6%). Statistically significant differences ($P<0.05$; χ^2) in frequency of antimicrobial resistance of isolates were noted for oxytetracycline, cephalothin and ampicillin across sources.

For humans who indicated that they have close contact with their pet dogs or cats, the frequency of resistance, 80.4% (45 of 56) to antimicrobial agents amongst *E. coli* isolates were similar to the frequency in isolates from pet animals, 82.6% (57 of 69) ($P>0.05$;

χ^2) as shown in Table 3. The frequency of resistance to oxytetracycline was statistically significant ($P<0.05$; χ^2) higher amongst pet isolates compared with humans (17.9%).

Table 1. Antimicrobial resistance of *E. coli* isolates from dairy farms in Waller field and Carlsen field

Farming area	N° of *E. coli* isolates tested	N° (%) of isolates resistant[1]	N° (%) of isolates resistant to:							
			STREP[2]	OXT	CF	AMP	SXT	CN	CIP	ENRO
Waller field	240	168 (70.0)	155 (64.6)	55 (22.9)	42 (17.5)	31 (12.9)	6 (2.5)	1 (0.4)	1 (0.4)	1 (0.4)
Carlsen field	260	217 (83.5)	208 (80.3)	67 (25.8)	78 (30.0)	49 (18.8)	13 (2.6)	6 (2.3)	0 (0.0)	0 (0.0)
Total	500	385 (77.0)	363 (72.7)	122 (24.4)	120 (24.0)	80 (16.0)	19 (3.8)	7 (1.4)	1 (0.2)	1 (0.2)

[1]Resistant to one or more antimicrobial agent; [2]STREP-Streptomycin, OXT-Oxytetracycline, CF-Cephalothin, AMP-Ampicillin, SXT-Sulphamethoxazole/Trimethoprim, CN-Gentamycin, CIP-Ciprofloxacin, ENRO-Enrofloxacin

Table 2. Frequency of resistance of *E. coli* isolates from both farming areas to antimicrobial agents by source of sample

Source	N° of *E. coli* isolates tested	N° (%) of isolates resistant[1]	N° (%) of isolates resistant to:							
			STREP[2]	OXT	CF	AMP	SXT	CN	CIP	ENRO
Cow	204	156 (76.5)	145 (71.4)	31 (15.2)	59 (28.9)	23 (11.3)	1 (0.5)	2 (1.0)	0 (0.0)	0 (0.0)
Calf	115	83 (72.2)	80 (69.6)	37 (32.2)	13 (11.3)	13 (11.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Dog	72	56 (77.8)	52 (72.2)	29 (40.3)	17 (23.6)	16 (22.2)	4 (5.6)	2 (2.8)	1 (1.4)	1 (1.4)
Human	60	48 (80.0)	45 (75.0)	11 (18.3)	15 (25.0)	18 (30.0)	7 (11.7)	1 (1.7)	0 (0.0)	0 (0.0)
Water	32	26 (81.3)	25 (78.1)	11 (34.4)	10 (31.3)	6 (18.8)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Milk	14	13 (92.9)	13 (92.9)	1 (7.1)	4 (28.6)	2 (14.3)	1 (7.1)	0 (0.0)	0 (0.0)	0 (0.0)
Cat	3	3 (100.0)	3 (100.0)	2 (66.7)	2 (66.7)	2 (66.7)	0 (0.0)	2 (66.7)	0 (0.0)	0 (0.0)
Total	500	385 (77.0)	363 (72.6)	122 (24.4)	120 (24.0)	80 (16.0)	13 (2.6)	7 (1.4)	1 (0.2)	1 (0.2)

[1]Resistant to one or more antimicrobial agent; [2]STREP-Streptomycin, OXT-Oxytetracycline, CF-Cephalothin, AMP-Ampicillin, SXT-Sulphamethoxazole/Trimethoprim, CN-Gentamycin, CIP-Ciprofloxacin, ENRO-Enrofloxacin

Table 3. Antimicrobial resistance of *E. coli* isolates from humans according to degree of pet contact

Degree of pet contact[4]	Source of isolates[3]	N° of *E. coli* isolates tested	N° (%) of isolates resistant[1]	No (%) of isolates resistant to:							
				STREP[2]	OXT	CF	AMP	SXT	CN	CIP	ENRO
Very close	Pets	69	57 (82.6)	53 (76.8)	30 (43.5)	19 (27.5)	17 (24.6)	4 (5.8)	4 (5.8)	1 (1.4)	1 (1.4)
	Human	56	45 (80.4)	43 (76.8)	10 (17.9)	15 (26.8)	18 (32.1)	7 (12.5)	1 (1.79)	0 (0.0)	0 (0.0)
Subtotal		125	102 (81.6)	96 (76.8)	40 (32.0)	34 (27.2)	35 (28.0)	11 (8.8)	5 (4.0)	1 (0.8)	1 (0.8)

[1]Resistant to one or more antimicrobial agent ; [2]STREP-Streptomycin, OXT-Oxytetracycline, CF-Cephalothin, AMP-Ampicillin, SXT-Sulphamethoxazole/Trimethoprim, CN-Gentamycin,CIP-Ciprofloxacin, ENRO-Enrofloxacin; [3]Dogs and cats; [4]In households with either very close or moderately close pet-human interaction

A total of 22 resistance patterns were observed but the predominant ones were AMP-CF-STREP-OXT (19 isolates), AMP-CF-STREP (19 isolates), AMP- STREP-OXT (16 isolates) and CF-STREP-OXT (14 isolates) as displayed in Table 4.

Table 4. Frequency of resistant patterns amongst isolates of *E. coli* from all sources

Resistant pattern	N° (%) of isolates[2]
[1]AMP, CF, STREP, OXT	19 (3.8)
AMP, CF, STREP	19 (3.8)
AMP, STREP, OXT	16 (3.2)
CF, STREP, OXT	14 (2.8)
AMP, CF, CN, STREP, OXT, SXT	4 (0.8)
AMP, CF, STREP, SXT	4 (0.8)
AMP, CF, STREP, OXT, SXT	3 (0.6)
AMP, CF, CN, STREP, OXT, SXT	2 (0.4)
AMP, CIP, CN, STREP, ENRO, OXT	1 (0.2)
CF, STREP, OXT, SXT	1 (0.2)
AMP, STREP, SXT	1 (0.2)
STREP, OXT, SXT	1 (0.2)
Others[3]	300 (60.0)
No resistance	115 (23.0)

[2]STREP-Streptomycin, OXT-Oxytetracycline, CF-Cephalothin, AMP-Ampicillin, SXT-Sulphamethoxazole/Trimethoprim, [1]AMP-Ampicillin, STREP-Streptomycin, SXT-Sulphamethoxazole/Trimethoprim, CN-Gentamycin, CIP-Ciprofloxacin, OXT-Oxytetracycline, CF-Cephalothin, ENRO-Enrofloxacin; [2] Based on 500 isolates; [3]A total of 10 patterns with 2 or less antimicrobial agents

Table 5. Antimicrobial resistance of *E. coli* isolates according to type of veterinary service used

Type of Veterinary service	Nº of *E. coli* isolates tested	Nº (%) of isolates resistant[1]	Nº (%) of isolates resistant to:							
			STREP[2]	OXT	CF	AMP	SXT	CN	CIP	ENRO
State[3]	483	374 (77.4)	351 (72.7)	119 (24.6)	117 (24.2)	79 (16.4)	13 (2.7)	7 (1.4)	1 (0.2)	1 (0.2)
Private	17	8 (47.1)	12 (70.6)	3 (17.6)	3 (17.6)	1 (5.9)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total	500	382 (76.4)	363 (72.6)	122 (24.4)	120 (24.0)	80 (16.0)	13 (2.6)	7 (1.4)	1 (0.2)	1 (0.2)

[1]Resistant to one or more antimicrobial agent; [2]STREP-Streptomycin, OXT-Oxytetracycline, CF-Cephalothin, AMP-Ampicillin, SXT-Sulphamethoxazole/Trimethoprim, CN-Gentamycin, CIP-Ciprofloxacin, ENRO-Enrofloxacin; [3]Government-employed veterinarians

The frequency of resistance to antimicrobial agents was compared with the use of veterinary services, as shown in Table 5. The frequency of resistance from farms serviced by State veterinarians 77.4% compared to those of private veterinarians 47.1% was statistically significant ($P<0.05$; χ^2). For each of the eight antimicrobial agents tested, resistance was higher amongst isolates of *E. coli* from farms serviced by state veterinarians compared with those who employed the services of private veterinarians.

A statistically significant difference ($P<0.05$; χ^2) in resistance was noted in isolates of *E. coli* recovered from cattle that had experienced episodes of diarrhoea within the last six months (66.6%) compared with isolates recovered from cattle that had experienced episodes at an interval of greater than 6 months (86.3%).

For calves, a statistically significant difference ($P<0.05$; χ^2) was observed in the overall frequency of resistance to antimicrobial agents amongst *E. coli* isolates from calves with no experience of diarrhoea (60.1%) within the last 1 year compared with isolates recovered from calves where diarrhoea was reported every 1 to 2 months (84.6%) and an interval of greater than 6 months (81.4%). The statistically significant difference in frequency observed was primarily due to frequency of resistance to oxytetracycline compared with other antimicrobial agents tested.

For *E. coli* isolates from humans, it was observed that 78.2% (43 of 55) were resistant to one or more antimicrobial agents for isolates recovered from humans where no diarrhoea was reported, compared with 100% (5 of 5) resistance in *E. coli* isolates obtained from human individuals where diarrhoea was reported every 1 to 2 months. The highest frequency of resistance was to streptomycin (75.0%) and the lowest, (0.0%) to the fluoroquinolones.

Table 6. Frequency of antimicrobial resistance amongst *E. coli* isolates in relation to presence of *E. coli* O157 serogroup and various virulence markers

Virulence marker/ gene	Detection method	N° of isolates tested	N° (%) of isolates resistant	N° (%) of isolates resistant to:							
				STREP[2]	CF	TE	AMP	CN	SXT	ENRO	CIP
E. coli O157 serogroup	Direct plating [b]	8	8 (100.0)	8 (100.0)	6 (75.0)	4 (50.0)	1 (12.5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
VT	Veroassay[c]	61	48 (78.7)	46 (75.4)	20 (32.8)	14 (23.0)	11 (18.0)	1 (1.6)	0 (0.0)	0 (0.0)	0 (0.0)
LT		26	25 (96.2)	24 (92.3)	4 (15.4)	6 (23.1)	10 (38.5)	0 (0.0)	1 (3.8)	0 (0.0)	0 (0.0)
VT1-gene	PCR[d]	6	4 (66.7)	4 (66.7)	2 (33.3)	2 (33.3)	1 (16.7)	1 (16.7)	0 (0.0)	0 (0.0)	0 (0.0)
VT2-gene		15	10 (66.7)	8 (53.3)	3 (20.0)	5 (33.3)	3 (20.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
VT1 and VT2-gene		4	2 (50.0)	2 (50.0)	0 (0.0)	1 (25.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Eae		7	4 (57.1)	4 (57.1)	2 (28.6)	2 (28.6)	1 (14.3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
LT-gene		3	3 (100.0)	0 (0.0)	1 (33.0)	2 (66.7)	2 (66.7)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Total	130	104 (80.0)	96 (73.8)	38 (29.2)	36 (27.7)	29 (22.3)	2 (1.5)	1 (0.8)	0 (0.0)	0 (0.0)

[a]Resistant to one or more antimicrobial agents; [b]Samples (faeces, milk, effluent) plated on MacConkey, EMB and SMAC and tested by slide agglutination *E. coli* 0157 serogroup antiserum; [c]Veroassay to detect VT and LT positive samples; [d]PCR to detect the presence of VT1 an sVT2 gene using 0157 multiplex: LT and eae genus using 5 toxin multiplex.

Of the *E. coli* isolates recovered from pets where no diarrhoea was reported, 78.6% (55 of 70) were resistant to one or more antimicrobial agents compared with a 100% (3 of 3) frequency of resistance found in pets where diarrhoea was reported every one to two months, The difference was, however, not statistically significant ($P>0.05$; χ^2). In the moderate category 100% (2 of 2) isolates were reported to be resistant to one or more antimicrobial agents. The highest frequency of resistance was noted to streptomycin, (80.0%), and the lowest was to the fluoroquinolones (1.3%).

Table 6 shows the frequency of antimicrobial resistance amongst *E. coli* isolates that were positive for *E. coli* O157 serogroup, VT-positive, *eae*-positive and LT-positive. Frequency of resistance was statistically significantly ($P<0.05$; χ^2) higher amongst LT-positive isolates than VT-positive isolates. For *E. coli* isolates positive for virulence genes, 66.7% (4 of 6) VT1-positive isolates, 66.7% (10 of 15) VT2- positive isolates, 50.0% (2 of 4) VT1- and VT2- positive isolates, 57.1% (4 of 7) *eae* gene positive isolates and 100.0% (3 of 3) LT- gene positive isolates exhibited resistance. The differences were not statistically significant ($P>0.05$; χ^2). For all 130 *E. coli* isolates tested that were positive for O157, VT gene, VT or LT-production, 104 (80.0%) exhibited resistance to one or more antimicrobial agents. Frequency of resistance was highest to streptomycin, 73.8% (96 of 130) and lowest to the fluoroquinolones, 0.0% (0 of 130).

Discussion

It may be therapeutically significant that 77% of all isolates tested across sources were resistant to one or more antibiotics. This is because all the antimicrobial agents tested here, with the exception of the fluroquinolones, are currently commonly used in human and veterinary therapeutics in Trinidad. Across sources, resistance was highest to streptomycin and lowest to the fluroquinolones. This is consistent with studies done globally where it is known that distribution of antimicrobial resistance phenotypes are dependent upon use patterns of antimicrobial agents (LANZ et al., 2003). The finding that the highest frequency of resistance was to streptomycin (72.2%), in the present study reflects the common general use of streptomycin in veterinary practice. NORMAND et al. (2000) in Scotland had reported that resistance to streptomycin was due to an increase in drug usage or other causes leading to the development of selection pressures inducing streptomycin resistant bacteria. In Switzerland, it was also reported that resistance to streptomycin and tetracycline was most common and that an increasing resistance to cephalothin and enrofloxacin reflected the frequency of use of the antimicrobial agents (LANZ et al., 2003).

Although farmers in both farming areas did not admit to personally administering antimicrobial agents to their dairy animals, it is a general perception that they actually do so. Penicillin, along with other antimicrobial agents, is easily acquired by farmers from agricultural stores around the country, without restrictions. The farmers' problems are compounded by the fact that State veterinary services are available from 8:00 a.m. to 4:00 p.m. on weekdays only. Of concern is the fact that frequent and uncontrolled use of antimicrobial agents by farmers, particularly streptomycin and tetracycline, can induce antimicrobial resistance in humans through contact with cattle or their products. The comparatively very low frequency of resistance displayed to the fluoroquinolones (0.2%) may be a reflection of its uncommon use in the local dairy industry. The fact that all the isolates of *E. coli* that were resistant to fluoroquinolones originated from dogs

may be a reflection of their more frequent use in domestic animals compared to food producing animals.

Although cephalothin is not used in food-producing animals in Trinidad, a frequency of resistance of 24% was observed which is comparable to the frequency detected for oxytetracycline in the local dairy farms. This suggests gene linkage on plasmids as indicated by SCHROEDER et al. (2002). It has been postulated that general resistance to other beta-lactam antimicrobials results from use of beta-lactams such as ampicillin, which are more commonly used in veterinary and human medicine (SCHROEDER et al., 2002). This may explain the high frequency of resistance to cephalothin, which although not used in treatment protocols, is a beta-lactam antimicrobial. It has been demonstrated that the majority of multiple antimicrobial resistant phenotypes are due to acquisition of external genes that provide resistance to an entire class of antimicrobials (WHITE and McDERMOTT, 2001).

The frequency of resistance to oxytetracycline (32.2%) found in dairy-calves in the present study is considerably lower than the 77.7% earlier reported (ADESIYUN and KAMINJOLO, 1992) for isolates of *E. coli* from dairy-calves across livestock farms in Trinidad and Tobago. The lower frequency of resistance to oxytetracycline in the present study may indicate a reduction in the local use of the antibiotic in calves. It is known that Neorease^{R}, an anti-diarrhoeal agent (which does not contain oxytetracycline) is composed of neomycin, sulphamethazine and sulphathiazole, is the most frequently used in treatment in diarrhoeic calves in Trinidad. The use of an alternative antibiotic could explain the reduced resistance to oxytetracycline which was previously the antibiotic of therapeutic choice. The general high frequency of resistance amongst *E. coli* isolates from calves supports the findings of other studies that resistant bacteria are known to occur commonly in younger animals (BERGE et al., 2005; KHACHATRYAN et al., 2004).

ADESIYUN et al. (1997a) reported a 79.8% frequency for resistance to antimicrobial agents amongst *E. coli* isolates recovered from dogs sampled at a veterinary clinic using similar antimicrobial agents, which is comparable to the current study where 77.8% of isolates were resistant to 1 or more antimicrobial agents. In the latter study, the highest prevalence of resistance was to tetracycline (59.2%) in apparently healthy clinic dogs compared to a prevalence of 40.3% found in the present study.

The high prevalence of resistance (59.2%) to tetracycline reflects its common use in companion animal practice (ADESIYUN et al., 1997a). SEEPERSADSINGH (2003) reported a prevalence of 23.2% for antimicrobial resistance in *E. coli* isolates from farm dogs with the highest frequency of resistance (69.2%) being exhibited to cephalothin. For dogs sampled from all sources (farm, hunting, household and clinics) in the same study, the highest antimicrobial resistance was to cephalothin (30.2%) and the lowest to fluoroquinolones (0.5%). Although norfloxacin was evaluated in that study and enrofloxacin in the present

study, it is known that resistance to one fluoroquinolone confers resistance to the entire class (ORDEN et al., 1999).

The highest frequency of resistance amongst *E. coli* isolates from humans was to streptomycin (75%), followed by ampicillin (30%), cephalothin (25%) and tetracycline (18.3%). BONTEN et al. (1990) reported that the frequency of resistance of *E. coli* isolates recovered from humans in the Netherlands was highest to ampicillin (76%) followed by tetracycline (47%). The considerably higher prevalence of resistance to tetracycline reported by BONTEN et al. (1990) compared with the present study, may reflect, amongst other factors, a difference in the use of tetracycline in both humans and animals in both countries. SCHROEDER et al. (2002) reported that 50% of isolates of *E. coli* from humans were resistant to ampicillin, cephalothin, tetracycline or streptomycin. They also reported that 75% of the isolates resistant to ampicillin were also resistant to streptomycin and tetracycline, suggesting that resistance genes for these drugs are linked on plasmids.

Effluents are on-farm reservoirs of resistant bacteria that provide a potential source for resistant gene-transfer between bacteria and farm sources. The high frequency of resistance to streptomycin (78.1%) and to tetracycline (34.4%) by isolates from effluents is a reflection of the faecal contamination resulting from farm run-offs. The frequency of resistance to streptomycin and tetracycline is supported by studies done by SAYAH et al. (2005) in the United States, where it was suggested that resistance patterns may have been representative of contamination of surface water with antimicrobial resistant bacteria.

For pets in close contact with humans, the similarity in frequency resistance to antimicrobial agents between *E. coli* isolates recovered from both pets and their owners has zoonotic implications, as the findings suggest exchange of *E. coli* strains between pet animals and their owners. In Trinidad and Tobago, ADESIYUN et al. (1997c) reported an exchange of *Staphylococcus aureus* between animal handlers and their milking cows.

The antibiotic resistance patterns detected in the current study are comparable to those reported elsewhere. BERGE et al. (2003) reported a prevalence of resistance of 79.5% for isolates with single or double resistance patterns to tetracycline and sometimes ampicillin, based on cluster analysis, to detect antibiotic resistance patterns in *E. coli* from faecal samples of calves. BERGE et al. (2005) suggested that the presence of highly resistant *E. coli* strains isolated in calves not exposed to antibiotics may be a result of individual calf therapy providing enough selection pressure to maintain a resistant gene pool in the population. The authors also suggested that, even in the absence of multiple antibiotic use, *E. coli* multiple resistant strains survive well due to efficient iron-scavenging mechanisms (siderophores) or increased adhesion capacity that enhances colonization and spread of resistant genes.

Resistance to streptomycin was highest in both isolates from pet animals, livestock and humans where diarrhoea occurred either frequently, or not at all. This is reflective of

the common use of this antimicrobial in both human and veterinary medicine. Of interest was the occurrence of resistance to cephalothin in cattle, which may be due to acquisition of external genes that provide resistance to an entire class of antimicrobials (WHITE et al., 2001). It may also be attributed to resistance to the antimicrobial agent by Darwinian selection pressures in the environment (NORMAND et al., 2000).

The occurrence of antimicrobial resistance in isolates with phenotypic and genotypic characteristics is of concern. Amongst *E. coli* isolates from all sources, resistance to streptomycin was most predominant. It was hardly a surprise to observe that in all cases, the highest frequency of resistance across genotypic markers (*E. coli* O157 serogroup, VT, LT and *eae* positive isolates) was to streptomycin (73.8%) and non-existent to the fluoroquinolones. In a study conducted by KHAN et al. (2002) in India, resistance, regardless of source, was highest to tetracycline (23.8%) and streptomycin (14.3%) in VTEC isolates from diverse sources and in another study conducted by BETTELHEIM et al. (2003) a much higher level of resistance (15.5%) was found among VTEC than among-non-VTEC isolates (32.9%), regardless of source. This is significant, as these strains pose therapeutic implications due to their virulence characteristics. There is, however, a dearth of information available on the antimicrobial resistance of VTEC strains (KLEIN and BÜLTE, 2003).

In conclusion, the high prevalence of resistance to streptomycin and oxytetracycline agents amongst *E. coli* isolates, particularly those with virulence markers, may have therapeutic and zoonotic implications. The relatively high prevalence of resistance to streptomycin and oxytetracycline suggests that there must be more prudent use of antimicrobial agents by farmers.

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SAŽETAK

Presječno istraživanje provedeno je na 50 farmi mliječnih krava u Trinidadu. Uzorci izmetina krava, teladi i ljudi, te rektalni obrisci pasa bili su pretraženi na prisutnost bakterije *E. coli*. Pretraženi su bili i skupni uzorci mlijeka iz spremnika za mlijeko te uzorci iz mljekovoda. Za određivanje otpornosti na osam različitih antimikrobnih tvari difuzijskim je postupkom bilo pretraženo 500 izolata. Za dokazivanje tvorbe verotoksina i određivanje termolabilnosti rabljena je VERO stanična kultura, dok su odabrani izolati bili pretraženi lančanom reakcijom polimerazom za dokazivanje gena za verotoksin. Na području Waller je 70,0% (168 of 240) izolata *E. coli* bilo otporno na jedno ili više antimikrobnih tvari, a na području Carlsen 83,5% (217 of 260) ($P<0.001$; $\chi 2$). Učestalost otpornosti izolata bila je najveća prema streptomicinu (72,7%), a najmanja prema fluorokinolonima (0,2%). Od 130 pretraženih izolata serološke skupine O157, koji su sadržavali gen za verotoksin, proizvodili ~~verotoksin ili termolabilni toksin otporna su bila 104 (80,0%) izolata. Otpornost izolata bakterije *E. coli* koji~~ su posjedovali markere virulencije mogla bi predstavljati problem u liječenju na farmama mliječnih krava na Trinidadu.

Ključne riječi: antibiogram, *E. coli*, mliječne farme, Trinidad