Accepted Manuscript

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Authors: Ana Verónica Martínez-Vázquez, Gildardo Rivera-Sánchez, Krystal Lira Méndez, Miguel Ángel Reyes-López, Virgilio Bocanegra-García

PII: S2213-7165(18)30044-4
DOI: https://doi.org/10.1016/j.jgar.2018.02.016
Reference: JGAR 611

To appear in:

Received date: 27-6-2017
Revised date: 16-2-2018
Accepted date: 20-2-2018

Please cite this article as: Ana Verónica Martínez-Vázquez, Gildardo Rivera-Sánchez, Krystal Lira Méndez, Miguel Ángel Reyes-López, Virgilio Bocanegra-García, Prevalence, antimicrobial resistance and virulence genes in Escherichia coli isolated from retail meats in Tamaulipas, México. (2010), https://doi.org/10.1016/j.jgar.2018.02.016

Prevalence, antimicrobial resistance and virulence genes in *Escherichia coli* isolated from retail meats in Tamaulipas, México.

Running Title: *E. coli* characterization in meat from Mexico

Ana Verónica Martínez-Vázquez, Gildardo Rivera-Sánchez, Krystal Lira Méndez, Miguel Ángel Reyes-López and Virgilio Bocanegra-García*

Centro de Biotecnología Genómica, Instituto Politécnico Nacional

Blvd. del Maestro and Elías Piña, Reynosa, Tamaulipas, Mexico, 88710

*Corresponding author: Virgilio Bocanegra García.

Centro de Biotecnología Genómica, Instituto Politécnico Nacional

Blvd. del Maestro and Elías Piña, Reynosa, Tamaulipas, Mexico, 88710. E-mail: vbocanegra@ipn.mx . Phone/Fax: +52 (899) 9243627 ext. 87753

Highlights

- High percentage of the strains harbored virulence factors (*hly*A, *stx*1 and *stx*2)
- Multidrug resistance was detected in 92.4% of strains
- Those strains may play an important role in disseminating drug resistance
- With all the above, those strains may represent a public health risk.

ABSTRACT

Objective

The aim of this work was to determinate the prevalence of *Escherichia coli* and its resistance to antimicrobials and the presence of virulence genes in retail samples of beef and pork in several locations in Tamaulipas.

Methods

In this work, a total of 106 samples (beef and pork) collected from August 2013 to March 2014, were analyzed to detect *Escherichia coli* and then analyzed for virulence, antibiotic resistance gene detection, and tested for susceptibility to 16 antimicrobials.

Results

One hundred fifty-eight *Escherichia coli* isolates were obtained and of these, 1.8% harbored *stx*1; *stx*2 and *hly*A was detected in 17.7% and 21.5% of isolates, respectively. High-resistance phenotypes were observed in almost all of the isolates since 92.4% showed a multi-resistant phenotype with resistance to cephalothin 92%, ampicillin 92%, cefotaxime 78%, nitrofurantoin 76% and tetracycline 75%. *tet*A and *tet*B were detected in 56% of isolates, *str*A in 9.6%, *aad*A in 17%, and *aac*(3)-IV in only 0.6% of strains.

Conclusions

Based on these results, it can be concluded that retail beef and pork meat, might play a role in the spread of antibiotic resistant *Escherichia coli* strains in our region.

Keywords: meat, antimicrobial resistance, virulence factors, *Escherichia coli,* Mexico

1. Introduction

Foodborne diseases (FBD) typically present as diarrheic episodes, affecting 550 million patients every year. The World health organization (WHO) annually estimates that 1 in 10 people become sick by consuming contaminated foods Many of these diseases are frequently associated with consumption of contaminated meat that has not been adequately cooked [1], as has been previously reported, the detection of pathogens such as such as *Salmonella* sp, and *Escherichia coli* [2, 3, 4, 5]. In addition to the above, in recent times, *E. coli* has also taken relevance as a study model for drug resistance dissemination in bacterial populations and as an indicator of indiscriminate antibiotic use selective pressure in animal production [6, 7, 8, 9] making it a reference model in worldwide monitoring programs of drug resistance [2], which may help to establish strategies to reduce the risk to the population [10]. In Mexico, prevalence studies of *E. coli* in meat are scarce; therefore, we do not have readily available information about the level of drug resistance and the distribution of virulence genes [11, 12, 13, 14]. The aim of this work was to determinate the prevalence of *E. coli* and its resistance to antimicrobials and the presence of virulence genes in retail samples of beef and pork in several locations in Tamaulipas.

2. Material and methods

2.1 Sample collection

From August 2013 to March 2014, 106 meat samples, including 54 beef samples and 52 pork samples were purchased randomly from 55 supermarkets and retail stores (butcheries) located in eleven cities of Tamaulipas, Mexico. From each city, 5 supermarkets were randomly sampled. In each store one beef ground sample and one pork ground sample were purchased randomly, in packing from about 500 g presentation. All samples collected were

aseptically manipulated, labeled and store individually in ice for transport to the laboratory in the Centro de Biotecnología Genómica.

2.2 Isolation and identification of *Escherichia coli*

Microbiological analysis was done according to the national Mexican standard for pathogen detection in foods, NOM-210-SSA1-2014 [15]. Twenty-five gram portions were obtained for each sample, and homogenized for 2 min. After homogenization, samples were cultured on plates of Eosin Methylene Blue (EMB) agar. After 18–24 h of incubation at 37°C, the presumptive colonies with characteristics corresponding to *E. coli* morphology were selected. From each sample, six colonies were then individually inoculated in tryptic soy agar (TSA, Difco) and incubated for 24 h a 37°C, in order to obtain a pure culture (six isolates per beef sample and 6 isolates per pork sample). Standard biochemical tests were applied to confirm the identity of *E. coli* (lactose fermentation, citrate metabolism, methyl-red-Voges-Proskauer, urease production and indole production test).

2.3 Virulence gene detection

Bacterial DNA for PCR was obtained by suspending colonies of bacteria from a 24 h culture from Tryptic soy agar plates (TSA, Difco) in 500 µL of sterile water and boiling at 95ºC for 15 min, followed by centrifugation at 13,000 g for three minutes. PCR analyses were performed using specific primers to the major Enterohemolysin/Shiga toxin-producing *E. coli* (EHEC/STEC) virulence genes that encode Shiga toxin *stx*1-*x*2 and *hly*A as showed in table 1[16] . The PCR reaction mixture contained a final concentration of buffer 1X, $MgCl_2$ 25 mM, dNTPs 10 mM, primer 10 mM, Taq DNA polymerase 5 U and sterile water in a final volume of 25 µL. PCR amplification conditions were as follows:

initial denaturation at 95ºC for 1 min, followed by 30 cycles of denaturation at 95ºC for 45 s, annealing at 53ºC for 45 s, and extension at 72ºC for 45 s and a final cycle of amplification at 72ºC for 7 min. PCR products were evaluated in 2.5% agarose gel with sybr gold at 100 V for 45 min. Negative controls (samples without a DNA template) and positive controls (samples with DNA from the collection of the National Polytechnic Institute) were included in all PCR assays. The DNA bands were visualized and photographed under UV light.

2.4 Antimicrobial susceptibility testing

Antimicrobial susceptibility was tested by the agar disc diffusion method, according to the Clinical and Laboratory Standards Institute guidelines [17]. The antimicrobials used included the following 16 agents: tetracycline (TET, 30 µg), amoxicillin/clavulanic acid (AMC, 30 µg), ciprofloxacin (CIP, 5 µg), amikacin (AK, 30 µg), ampicillin (AM, 10 µg), levofloxacin (LEV, 5 µg), cephalothin (CF, 30 µg), cefotaxime (CTX, 30 µg), ceftriaxone (CRO, 30 µg), chloramphenicol (CL, 30 µg), gentamicin (GE, 10 µg), netilmicin (NET, 30 µg), nitrofurantoin (NF, 300 µg), cefepime (FEP, 30 µg), trimethoprim/sulfamethoxazole (SXT, 25 µg) and streptomycin (STR, 30 µg). *E. coli* strains were evaluated based on the diameter of the clear zone of inhibition around each antimicrobial disk, which was measured in millimeters. The results were interpreted in accordance with criteria provided by the CLSI (Clinical and Laboratory Standards Institute), and were classified as susceptible, intermediate or resistant. These antimicrobials are representative of the major classes of antimicrobial drugs that are important to both veterinary and human medicine. *S. aureus* strain ATCC 29213 and *E. coli* strain ATCC 25922 were used as controls.

2.5 Detection of antimicrobial resistance genes

The presence of genes associated with tetracycline (*tet*A and *tet*B), β-lactam (bla_{TEM}, bla_{NDM-1}, bla_{SHV}) and aminoglycoside (*str*A, *aad*A, *aac*(3)-IV) resistance were detected by PCR assay [18, 19]. PCR was done on bacterial lysates as described before. PCRs were performed in 25 μL reaction mixture containing buffer 1X, $MgCl_2$ 25 mM, dNTPs 10 mM, primers 10 mM, Taq DNA polymerase 5 U and sterile water in a final volume of 25 μL. PCR amplification conditions were 95ºC for 1 min, 30 cycles at 95ºC for 45 s, 59-42ºC for 45s, and 72ºC for 45 s with a final amplification cycle at 72ºC for 7 min. Appropriate positive and negative controls were included in each PCR run. PCR products were electrophoresed in 2.7% agarose gel with syber gold at 100 V for 45 minutes.

2.6 Statistical analysis

Data were analyzed by using SPSS version 24.0 software (SPSS, IBM, Somers, NY, USA). Univariate analysis was performed for calculation of difference in prevalence by using the χ^2test. The level of significance was set at $P < 0.05$. Descriptive statistics (estimation of proportions) were used to summarize the prevalence of *E. coli* and antimicrobial sensitivity patterns of the isolates.

3. Results

3.1 *E. coli* Prevalence

In total, 636 strains were isolated out of the 106 meat samples (324 stains from beef samples and 312 strains from pork samples) from the 11 cities of Tamaulipas included in this work. From the 106 total meat samples collected, 59 (55.6%; 59/106) were positive for *Escherichia coli*, 29/59 (49.1%) were isolated from beef samples and 30/59 (57.6%) were

isolated from pork samples. From those 636 strains, 158 were confirmed as positive for *E. coli*. We identified 158 strains as *E. coli*, being 74/158 strains (46.8%) from 29 beef samples and 84/158 strains (53.1%) from 30 pork samples. The overall prevalence in the 11 cities was 24.8% (158/636 strains). Most of the *E. coli* isolates were obtained from Nuevo Laredo with 5/5 beef samples and 4/5 pork samples being positive for *E. col*i, representing the 8.4% of all positive samples (9/106) and 24.6% of all positive strains (39/158) ($p>0.05$, table 1). The city with the lowest percentage of *E. coli* isolates detected was Valle Hermoso with 2/4 beef samples and 0/3 pork samples, with 0.6% samples positive (2/106) and 2.5% of all positive strains (4/158) as showed in Figure 1 and Table 1.

3.2 Virulence gene detection

In all the 158 *E. coli* strains, the presence of the virulence genes, *stx*1, *stx*2 and *hly*A, was tested by PCR analysis; 41.1% (65/158) were positive for one of these genes. Of these 65 strains positive for virulence genes, 32 were from beef and 33 were from pork meat samples.

In 1.8% of the strains tested *stx*1 was detected; in 17.7% were positive for *stx*2 and *hly*A was the most prevalent being detected in 21.5% of strains. Only three strains contained both *stx*2 and *hly*A genes. None of the strains contained all the three genes (Table 1).

3.3 Drug resistance and drug resistance-related gene detection

In the phenotypic resistance tests to antimicrobials, 92.4% of *E. coli* strains were resistant to at least 4 different antimicrobials (Table 2 and 3). Most of these strains exhibited multi-drug resistance patterns to 7, 8, and 9 antibiotics, simultaneously (44%, n=77). One hundred and thirty-three different phenotypic resistance patterns were detected (Table 4).

Seventy-three strains came from beef samples and 85 strains from pork samples. Of these 158 *E. coli* strains, 91.7% (145/158) were resistant to CF and 90.5% (143/158) to AM, followed by 77.8% (123/158) resistant to CTX, 75.9% (120/158) to NF, and 67.7% (107/158) resistant to TET. On the other hand, 93.0% (147/158) were susceptible to NET, 90.5% (143/158) to CIP, 87.3% (138/158) to AK, and 81.6% to GE (129/158). We did not founded significant statistical associations between resistance phenotypes and meat type (Table 2). The presence of genes related to antibiotic resistance was also analyzed. Of the 107 *E. coli* strains resistant to TET (107/158), only in 60 (56.0%) one or both of the tested genes (*tet*A and *tet*B) were detected (Table 3). Of the strains resistant to STR (62/158), 6 had only *str*A (9.6%), 11 had *aad*A (17.7%), and 21 (33.8%) had both (*str*A and *aad*A). In isolates from beef, we detected the presence of one or both genes in 13/23 (56.5%) and in isolates of pork samples, we detected one or both genes in 25/29 (64.1%). In the GE resistant strains (29/158), *aac*(3)-IV was detected in only one strain. However in the strains resistant to STR, *aac*(3)-IV was detected in 2 isolates (3.2%), one from beef samples and one from pork samples. In the strains resistant to AM (143/158, 90.5%) and AMC (87/158, 55.0%), bla_{TEM} was the most prevalent, being present in 18 AM-resistant strains (18/143, 12.5%), and in 12 AMC-resistant strains (12/87, 13.7%). Only in 3 strains from beef samples resistant to AM (3/64) and AMC (3/37), bla_{SHV} was detected, and none of the strains from pork had it. On the other hand, $bla_{\text{NDM-1}}$ was not detected in strains from beef samples but it was present in 9 strains from pork samples (9/158, 5.6%).

1. Discussion

According to our results, we observed a greater prevalence of *E. coli* in the cities from Northern Tamaulipas, bordering with the United States. There are no previous reports in

this area so we cannot compare results with the present study. As far as we know, it is the first work done in this area of Tamaulipas. The presence of *E. coli* in retail meat indicates a low sanitary quality management and a potential risk to consumers´ health. Although it is considered that cooking meat destroys the *E. coli* that might be present, situations such as undercooking, low handler's hygiene or cross contamination of cooked food with raw meat or surfaces or utensils in contact with raw meat, can lead to further distribution of *E. coli* strains. The presence of a high quantity of *E. coli* can indicate low quality practices, although it does not always represent a health risk, since *E. coli* strains comprise a varied group of pathogenic and non-pathogenic serotypes. Shiga toxin producing *E. coli* (STEC) and enterohemorrhagic *E. coli* (EHEC) are strains that are considered a high health risk because they can cause diarrhea and serious conditions such as uremic hemolytic syndrome (UHS); in some cases, they can even cause death [20]. A common characteristic of all EHEC strains is the production of an EHEC-specific plasmid mediated hemolysin encoded by *hly*A [21] and at least one Shiga-like toxin (encoded by *stx*1 or *stx*2) [22]. Livestock is considered a reservoir for STEC strains, with the possible route of transmission to humans being by beef contaminated with fecal matter at some point in the processing routes [23, 24]. In several countries, STEC have been detected in beef and pork retail products (in addition to other beef products), by detecting *stx*1 and *stx*2. In the samples included in this work, we detected these genes alone or together in 19.6% of the isolated strains (31/158) (Table 1). This prevalence is similar to that reported by Minh et al. in Japan with 22% (24/270) [25], Park et al. from Korea with 17% [26], and Ateba et al. from South Africa with 23.7% [27]. In the case of each gene prevalence, we identified *stx*1and *stx*2 in 1.8% and 17.7% of the analyzed strains, respectively. This high predominance of *stx*2 has been observed in some other works such as Li et al. from China, with a prevalence of 4.9% and

27.6% of *stx*1 and *stx*2, respectively [28]. Similar results are in reports from Minh et al. with 6.6% and 14.8% of *stx*1 and *stx*2, respectively and Ateba et al. with 6.2% and 17.5% of *stx*1 and *stx*2, respectively [25, 27]. Those findings are relevant, because some epidemiological studies have indicated that strains carrying *stx*2 are potentially more virulent, and more frequently related to HUS, than those carrying *stx*1, or even those carrying both *stx*1 and *stx*2 [29]. Treatment of Enterohemorrhagic *E. coli* infections with antibiotics may worsen the illness, presumably by breaking up the bacteria with the release of more toxins and increased toxin production [23, 31]. However, early administration using some antimicrobials is effective [32]. Unfortunately, inappropriate ways of antimicrobial uses have contributed to the increase in antimicrobial resistance [33, 31] and have the ability to transfer antibiotic resistance to others, posing a challenge in the treatment of infectious diseases.

Of all analyzed strains, 146/158 were resistant to 4 and up to 9 antibiotics. This drug resistance may be considered high in comparison with similar reports from other places. For instance Sheikh et al. from Canada (with pork, beef, poultry and turkey samples) and Llorente et al. from Buenos Aires (with beef samples), reported a multi-drug resistance prevalence of 28.1 and 27.8%, respectively [2, 34]; both of them quite below our prevalence (92%). In the United States, Tadesse et al. made a review from 1950 to 2002 (of human and food samples from beef, pork, poultry) [35] finding a prevalence of multi-drug resistance of 54% (59.1% in beef, 53.7% in pork). Similar findings were reported for Skockova et al. from the Check Republic (with samples of beef, pork, poultry and deer) with a prevalence of 45.2% of multidrug resistance [36], that even that are higher prevalence, are still lower in comparison with our findings (92%). Looking for similar reports from Mexico, we could only find the work of Canizalez et al. from Sinaloa, being

apparently the first report of this kind of studies in Mexico [37]. In this work, several different kinds of food, raw and processed were analyzed for drug resistance to 9 antibiotics and 66% of the *E. coli* strains resulted resistant to one or more antibiotics and 39.2% of the strains were multi-drug resistant. These prevalence findings are still low compared with ours of 92% drug resistance; however, we tested 16 antibiotics, and that may affect the comparison of the findings.

Tetracycline and ampicillin are antibiotics that are widely used in similar published works; thus, we can make some comparison of results, but on the other hand, CTX, NF and CF are not frequently included. Sheikh et al. reports a resistance to TE of 20.5% (16.4% in beef and 31.7% in pork) and to AM with 7.2% (5.5% in beef and 12.2% in pork) [2]. Tadesse et al. reported the most co-resistance to TET and STR with 29.7%, TET and AM with 18.8%, and TET, AM, STR, and sulfonamide with 19.9% [35]. On the other hand, Llorente et al. reported a resistance prevalence of 28.1% to AM, STR, AK y TET although they did not give information about resistance to each individual antimicrobial [30]. In the same way, Skockova et al. also report AM and TET as the antibiotics with the most resistant strains, 29% and 25.8%, respectively [32]. In Mexico in the report of Canizalez et al. they indicate that the main resistance in the strains was to TET (34%), CTX (30%) and AM (29%) [33]. Unfortunately, these percentages were estimated in general for all the food samples included in the study, so we cannot do a direct comparison with the results in our meat samples.

In our work, we found that *E. coli* had a high prevalence of strains resistant to CF, AM, CTX, NF and TET; therefore, it was of particular interest to us to look for the presence of genes related with drug resistance to these antimicrobials. Out of the 158 *E. coli* isolates, 107 (67.7%) were phenotypically resistant to TET, and 81 (75.7%) had one or both genes,

tet(A) and *tet*(B). One interesting finding is that out of the 81 isolates with *tet*(A) and/or *tet*(B), 6 (7.4%) had intermediate resistance to TET and 15 (18.5%) were susceptible to TET. Also, 60 (60/158, 37.9%) strains had phenotypic resistance to TET and had one or both TET resistance related genes. For beta-lactam-related antibiotics, we tested the strains resistant to AM and AMC for the presence of *bla*TEM, *bla*SHV and *bla*NDM-1. Out of the 143 isolates with phenotypic resistance to AM, 25 (17.4%) had at least one of the *bla* genes, and in this case we only detected *bla* genes in phenotypically resistant strains. However, when we sought for the presence of *bla* genes in strains resistant to AMC, out of the 86 phenotypically resistant, only 16 (18.6%) had the presence of one of the *bla* genes. In this case, we also find *bla* genes in 4 strains with intermediate resistance and 4 strains susceptible to AMC. With regard to aminoglycosides, the presence of the genes *str*A, *aad*A and *aac*(3)-IV was according to the phenotypic resistance to STR.

The presence of antibiotic resistance genes in *E. coli* strains and their effect in phenotypic resistance are the result of a complex dispersion system; in this instance, Schmid et al. reported ESBL *E. coli* isolates from farms in which beta-lactam antibiotics were not used, suggesting that the presence of such isolates may be due to the use of other different classes or antibiotics that may also select ESBLs strains as well [34]. According to Jacoby et al. [35], resistance determinants against aminoglycosides, tetracycline, sulfonamides, and cephalosporin, are often situated on the same plasmid. Plasmids and transposons that carry multi-antimicrobial resistance genes can also carry virulence, and metabolic functions; for example, Tn1691 specifies resistance to some antibiotics: streptomycin, sulfonamides, and chloramphenicol [36]. This could indicate that there are factors other than veterinary medicines leading to the retention of antibiotic resistances within cattle. Some authors

indicate that even the air may be a vehicle for the transfer of elements of genetic resistance to antibiotics in bacteria [37, 38, 39].

2. Conclusions

To our knowledge, this study is the first report on prevalence and drug resistance in *E. coli* strains from beef and pork samples. The *E. coli* prevalence was 24.8% by city, indicating a low sanitary quality management. Coupled with this, the presence of virulence factors in a high percentage of the strains (41%), and the high multidrug resistance detected to beta-lactams, aminoglycosides and tetracycline may represent a health risk for beef consumers, because of an inadequate handling of the meat.

Acknowledgments

VBG, GRS and MARL are COFAA and EDI scholarship recipients of the Instituto Politécnico Nacional and members of the National Researchers System (SNI).

Declarations

Funding: No funding

Competing interests: None

Ethical approval: Not required

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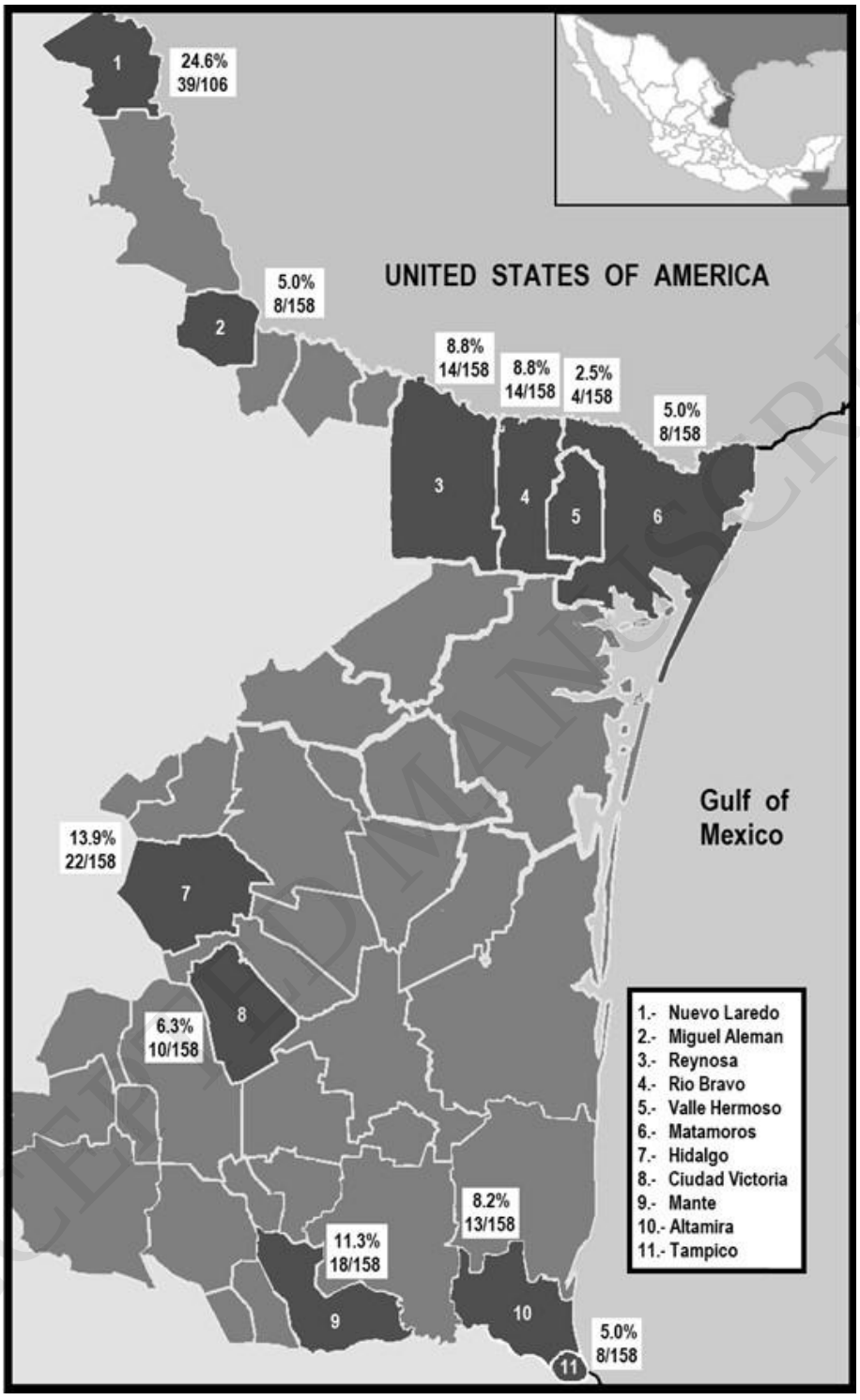

Figure 1. Prevalence of *Escherichia coli* isolated from meat samples in supermarkets of Tamaulipas. Percentage and number of all *E. coli* strains detected (158), is showed in each included city.

Table 1. Prevalence of *E. coli* strains and virulence factors detected.

City	*E. coli* by city/ 158 *E. coli* detected (%)		Other		p-value	*hly*A		*stx*1		*stx*2		*hlyA* + *stx*2		p-value
	No.	%	No.	%		No.	%	No.	%	No.	%	No.	%	
Altamira	13	8.2	47	9.8	>0.05	0	0	0	0	5	17.8	0	0	>0.05
Hidalgo	22	13.9	38	7.9	>0.05	0	0	0	0	13	46.4	0	0	**<0.05***
Laredo	39	24.7	21	4.4	**<0.05***	25	73.5	0	0	3	10.7	2	66.6	>0.05
Miguel Alemán	8	5.1	52	10.9	>0.05	2	5.8	0	0	0	0	0	0	>0.05
Mante	18	11.4	42	8.8	>0.05	4	11.7	0	0	2	7.1	1	33.3	>0.05
Matamoros	8	5.1	52	10.9	>0.05	0	0	1	33.3	2	7.1	0	0	>0.05
Rio bravo	14	8.9	46	9.6	>0.05	1	2.9	1	33.3	0	0	0	0	>0.05
Reynosa	14	8.9	46	9.6	>0.05	2	5.8	1	33.3	3	10.7	0	0	>0.05
Tampico	8	5.1	52	10.9	>0.05	0	0	0	0	0	0	0	0	>0.05
Victoria	10	6.3	50	10.5	>0.05	0	0	0	0	0	0	0	0	>0.05
Valle hermoso	4	2.5	32	6.7	>0.05	0	0	0	0	0	0	0	0	>0.05
No. Strains	158	100	478	100		34	100	3	100	28	100	3	100	

*Denotes statistical significance at P < 0.05

Table 2. Phenotypic resistance prevalence to antimicrobials in meat samples *E. coli* isolates.

Antimicrobial group	Microbial agent		Overall n=158 (%)	Beef n=74 (%)	Pork n=84 (%)	p-value
Aminoglycosides	Streptomycin	STR	62 (39.2)	23 (31.0)	39 (46.4)	>0.05
	Netilmicin	NET	11 (6.9)	5 (6.7)	6 (7.1)	>0.05
	Amikacin	AK	20 (12.6)	12 (16.2)	8 (9.5)	>0.05
	Gentamicin	GE	29 (18.3)	16 (21.6)	13 (15.4)	>0.05
Cefalosporin	Cephalothin	CF	145 (91.7)	67 (90.5)	78 (92.8)	>0.05
	Cefotaxim	CTX	123 (77.8)	54 (72.9)	69 (82.1)	>0.05
	Cefepime	FEP	88 (55.6)	40 (54.0)	48 (57.1)	>0.05
	Ceftriaxon	CRO	77 (48.7)	35 (47.2)	37 (44.0)	>0.05
β-Lactamans	Ampicillin	AM	143 (90.5)	64 (86.4)	79 (94.0)	>0.05
	Amoxicillin/ clavulanic acid	AMC	87 (55.0)	37 (50)	49 (58.3)	>0.05
Nitrofurantoin	Nitrofurantoin	NF	120 (75.9)	56 (75.6)	64 (76.1)	>0.05
Chloranphenicol	Chloranphenicol	CL	36 (22.7)	16 (21.6)	20 (23.8)	>0.05
Quinolones	Levofloxacin	LEV	41 (25.9)	20 (27.0)	21 (25)	>0.05
Sulfonamides	Sulfamethoxazole/trimethoprim	STX	76 (48.1)	30 (40.5)	46 (54.7)	>0.05
Tetracyclines	Tetracycline	TET	118 (74.6)	45 (60.8)	62 (73.8)	>0.05
Fluoroquinoles	Ciprofloxacin	CIP	15 (9.4)	6 (8.1)	9 (10.7)	>0.05
Statistically significant associations are P-values of < 0.05						

Table 3. Prevalence of genes related to drug resistance in *E. coli* isolates

Antimicrobial group	Phenotype resistance	Gene	Overall	
			No. isolates	(%)
Tetracyclines	Tetracycline (45/158)	*tet*(A)	26/45	57.7
		tet(B)	12/45	26.6
		tet(A) + *tet*(B)	22/45	48.8
Aminoglycosides	Streptomycin (62/158)	*str*A	6/62	9.6
		*aad*A	11/62	17.7
		*str*A + *aad*A	21/62	33.8
		aac(3)-IV	2/62	3.2
		*aad*A + *aac*(3)-IV	-	-
	Gentamicin (29/158)	*str*A	2/29	6.8
		*aad*A	6/29	20.6
		*str*A + *aad*A	11/29	37.9
		aac(3)-IV	-	-
		*aad*A + *aac*(3)-IV	1/29	3.4
β-lactams	Ampicillin (143/158)	bla_{TEM}	18/143	12.5
		bla_{NDM-1}	5/143	3.4
		bla_{SHV}	1/143	0.6
		bla_{TEM} + bla_{SHV}	1/143	0.6
	Amoxicillin/acid clavulinic (86/158)	bla_{TEM}	11/86	12.7
		bla_{NDM-1}	4/86	4.6
		bla_{SHV}	–	–
		bla_{TEM} + bla_{SHV}	1/86	1.1

Table 4. Phenotypic characteristic of multi-resistant of 28 *Escherichia coli* isolates of beef and pork samples collected in Tamauli pas.

Phenotype of resistance	Number of isolates	
	Beef (n=74)	**Pigs** (n=84)
STR TET	4	-
CF CTX AM NF	6	1
FEP CF CTX AM	1	-
STR CF STX TET	-	1
CF AM NF TET AMC	3	1
FEP CF CTX AM NF	2	-
FEP CF AM TET AMC	-	1
CF CTX STX AK AM AMC	1	-
FEP CF CTX AM CRO NF TET	1	1
FEP CF CTX STX AM CL NF	-	2
FEP CF CTX AM CRO NF AMC	3	3
FEP CF CTX STX AM NF TET AMC	-	1
FEP CF CTX STX AM CL NF TET	1	1
STR FEP GE CTX AK AM CRO TET	-	2
STR FEP CF CTX STX AM CRO NF TET	-	2
STR FEP CF CTX STX AM NF TET AMC	1	1
NET CF CTX STX AM CRO NF TET AMC	1	-
LEV CF CTX STX AM CRO CL NF TET AMC	-	1
LEV FEP CF CTX STX AM CRO NF TET AMC CIP	-	1
STR FEP CF CTX STX AM CRO CL NF TET AMC	1	1
FEP CF CTX STX AK AM CRO CL NF TET AMC	-	1
STR NET FEP CF GE CTX STX AM CRO NF TET AMC	-	1
STR FEP CF GE CTX STX AK AM CL NF TET AMC	1	-
LEV FEP CF GE CTX STX AK AM CL NF TET AMC	1	-
STR LEV FEP CF CTX STX AM CL NF TET AMC CIP	-	1
STR FEP CF GE CTX STX AK AM CRO NF TET CIP	1	-
STR LEV FEP CF CTX STX AM CRO CL NF TET AMC CIP	1	-
STR LEV NET FEP CF GE CTX STX AM CL NF TET CIP	1	-

STR=streptomycin; LEV=levofloxacin; NET=netilmicin; FEP=cefepime; CF=cephalothin; GE=gentamicin; CTX=cefotaxime; STX=sulfamethoxazole-trimethoprim; AM=ampicilin; CL=chloramphenicol; NF=nitrofurantoin; TET=tetracycline; CIP=ciprofloxacin.