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Research Article

Isolation And Molecular Identification Of Escherichia Coli And Salmonella Spp., From Pork, Beef And Chicken Meat Collected From Different Markets In Guaranda, Ecuador

FAVIAN BAYAS-MOREJÓN, RIVELIÑO RAMÓN-CURAY[1], MARÍA OLALLA-GARCÍA[2], BYRON HERRERA-CHÁVEZ[3]CESAR CARRASCO-RUANO[4]

[1]Centro de Investigación y Desarrollo Biotecnológico, Departamento de Investigación y Vinculación, Universidad Estatal de Bolívar, Facultad de Ciencias Agropecuarias, Recursos Naturales y del Ambiente, Guaranda EC020150, Ecuador

[2]Carrera de Enfermería, Facultad de Ciencias de la Salud y del Ser Humano, Departamento de Investigación y Vinculación, Universidad Estatal de Bolívar, CP: 020150, Guaranda-Ecuador

[3]Animal Production and Industrialization Research Unit, Engineering Faculty, Universidad Nacional de Chimborazo, Riobamba EC060150, Ecuador

[4]Hospital General Alfredo Noboa Montenegro, Guaranda EC020150, Ecuador

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ABSTRACT

The aim of the study was to evaluate the occurrence of E. coli and Salmonella spp. in Sixty-one meat samples (19 chicken, 22 pork and 20 beef), collected from the central markets in Guaranda, Ecuador. All samples were analysed by culture using specific methods for the two genus and the isolates obtained were confirmed by Polymerase Chain Reaction. By culture, 29 samples (47.59%) were positive for Escherichia spp. with 42 isolates, and 25 samples with 35 isolates for Salmonella spp. By PCR, 22 samples (36.07%) were positive for E. coli, and 23 samples (37.70%) for Salmonella spp. The highest prevalence rate of Escherichia coli was observed in pork and beef (16.39% each); A high prevalence of Salmonella spp. was detected in pork (18.03%) followed by beef (13.11%). This study highlights the importance of these pathogens and the need for further studies on its prevalence and distribution in different types of food.

Keywords: E. coli; Salmonella spp.; characterization; meats

INTRODUCTION

While the developing countries continue to struggle with the issue of food safety, that is, the amount of food sufficient for consumption by the growing population; there is another dilemma in these countries, it is estimated that more than 200 types of diseases caused by pathogens are foodborne, causing problems in vulnerable groups, therefore, guaranteeing healthy food is a challenge for public health (1). Among these pathogens are Escherichia coli and Salmonella spp. (2).

During recent years, E. coli has been identified as an emerging foodborne zoonotic pathogen worldwide and is associated with diseases such as: bacteraemia, gastroenteritis, abdominal pain, diarrhoea, nausea, vomiting and fever (3; 4).

Escherichia spp. have also been isolated from a variety of foods of animal origin, as chicken, beef and pork (5; 6).

Poultry species particularly act as an important reservoir of Escherichia spp. and as a major source of infection spread in human, may be associated with the consumption of contaminated raw or poorly cooked meat (7).

Salmonella spp. is one of the most important causal agents of foodborne diseases in developed and developing countries and is one of the main causes of acute bacterial enteritis in people worldwide, moreover, is commonly associated with livestock and poultry (8;5).

For the identification of Escherichia and Salmonella species have been developed, more rapid and with higher specificity than conventional identification methods, among which PCR and Multiplex PCR (9). The present study was designed to know the occurrence of Escherichia spp and Salmonella spp. in three types of meats in the Guaranda city by utilizing both bacteriological and molecular methods.

MATERIALS AND METHODS

Sample Collection

A total of 61 samples of three types of meats (19 chicken, 20 beef and 22 pork) have been collected from the local market of Guaranda

(Ecuador) and analysed between January and July 2017. All samples were transported and analysed within 04 h. to the Microbiology laboratory of the Universidad Estatal de Bolívar.

Sample Preparation and Bacterial Isolation

For isolation of Escherichia spp, 25 g of samples (meat) were aseptically inoculated in a 1:10 ratio in buffered peptone water (BPW) (Oxoid, UK) and homogenized separately for the culture of Salmonella spp and Escherichia spp.

For Salmonella spp. The dilution was incubated for 24 h at 37°C. After this, **ISO 579:2003/A1:2007** (10) guidelines were followed.

For E. coli isolation, TBX agar plates (Oxoid Ltd, England) were inoculated and incubated at 37°C for 24 h. Two randomly-selected colonies from each plate were transferred to Nutri Agar (Scharlau, Spain) and incubated to 37°C for 24 h. All the selected strains were analysed by Gram stain, to later assays, the strains were stored at -20°C.

Molecular Analysis

Five colonies of each strain grown on Nutrient agar to Escherichia spp. and TBX agar to Salmonella spp. were suspended in 500 μL of buffer TAE 1X and centrifuged at 16,000 g for 10 min at room temperature.

DNA extraction was performed using PureLink™ Microbiome DNA Purification Kit (Invitrogen, USA) following the manufacturer's instructions.

PCR Assay

The primers and PCR assay conditions used to confirm the identification of E. coli were previously described by **Lindsey et al. (9)**. To PCR in Salmonella was according to the method established by **Cheng et al. (11)**.

Electrophoresis of PCR Products

Five μL of amplification products, mixed with 2 µL of loading buffer Blue/orange 6X, loading Dye (Promega, USA) were separated in 1.5% agarose gels prepared with TAE buffer with 2 µl of SYBR Safe DNA gel stain (Invitrogen, USA), at 100 V for 40 min. Finally, the band´s sizes were visualized with a UV transilluminator. DNA from reference strains E. coli (ATCC 1053) and Salmonella arizonae (ATCC 13314) were used as positive controls.

RESULTS AND DISCUSSION

Cultural Isolation of Bacteria

Escherichia spp.

After culture analysis, Escherichia spp. showed 29/61 samples have the presence of the pathogen, with a total of 42 isolates. The suspect colonies showed typical white to whitish-gray color, small (2-4 mm) diameter. For the presence of Escherichia, beef and pork meat revealed a higher prevalence 10/61 positive samples of each, followed by chicken meat (Table 1). In contrast to these results, there is a work developed in Saudi Arabia by **Hessain et al. (12)**, where the detection level by culture was quite lower in relation to our work, with values of 2.97% (11 isolates). **Bindu Kiranmayi and Krishnaiah (13)**, detected E. coli by culture only in 3/100 meat samples. In Ecuador, there is an investigation with results quite similar to those obtained in our study: **Chiluisa et al. (14)**, by culture, identified E. coli in 40% of hamburger samples, 66.7% of shawarma samples and 53.3% of skewers.

Salmonella spp.

In the isolation of Salmonella spp. by culture, 25/61samples have the presence of the pathogen, with a total of 35 isolates. The pork meat revealed a higher prevalence of the pathogen, followed by beef and chicken (Table 1). Results with lower detection values than ours were obtained by **Ahmed et al. (15)**, 32 samples (21.3%) were positive by culture for Salmonella spp. In another study developed by **Shafini et al. (16)**, a total of 86 (27.6%) samples were positive for Salmonella spp. Also, a result much greater than ours was obtained by **Ifeanyichukwu et al. (17)**, with a prevalence of Salmonella spp. of 62% in poultry products. In Ecuador, there are few studies related to the detection and isolation of Salmonella spp. Thus, we have the work developed by **Vinueza-Burgos et al. (18)**, where, they analysed 388 batches of broilers randomly selected from Quito, obtaining a prevalence by culture of 16.0%.

Table 1: Samples detected with Escherichia coli and Salmonella spp. by culture and PCR

Type of sample	Number of sample	N° of sample detected by culture		N° of sample identifacted by PCR	
		E. coli	Salmonella spp.	E. coli	Salmonella spp.
Chicken	19	9 (14.76%)	6 (9.84%)	6 (9.83%)	6 (9.84%)
Beef	20	10 (16.39%)	8 (13.11%)	6 (9.83%)	7 (11.47%)
Pork	22	10 (16.39%)	11 (18.03%)	10 (16.39%)	10 (16.39%)

Total	61	29 (47.54%)	25 (40.985)	22 (36.07)	23 (37.70)

Identification of Escherichia coli and Salmonella spp. Isolates by PCR

In the first PCR to E. coli detection, 34 isolates (14 isolates/10 samples from pork; 11 isolates/6 samples from chicken and 9 isolates/6 samples from beef) were positive to E. coli, 36,07% positives simples (Table 1); in these samples, a characteristic band of 212-pb was obtained (Figure 1). Our result was similar to that obtained by **Jiménez et al**. (**19**), with 31.5% of meat analysed, in the same way as that obtained by **Hessain et al**. **(11)**, who detected E. coli in 27.27% of the meats analysed.

In the second PCR to Salmonella spp detection, 32 isolates (12 isolates/10 samples from pork; 11 isolates/7 samples from beef and 9 isolates / 6 samples from chicken) were positive for Salmonella spp, (37.70%) (Table 1); in the positive isolates a characteristic band of 262-pb was obtained (Figure 2). Similar results were obtained by **Trongjit et al**. **(20)** in Cambodia, where 47% of the samples of pork and chicken were positive for Salmonella spp. by PCR. On the other hand, **Naik et al**. (**21**), obtained a detection value of Salmonella in meat less than 10%.

In our study, it is important to emphasize that in three beef samples (4.91%) and five pork samples (8.20%), it was possible to obtain isolates of E. coli and Salmonella spp. of the same sample. While chicken meat was isolated from different samples. In the first case it was possibly due to cross contamination (the meats on the market were in the same container).

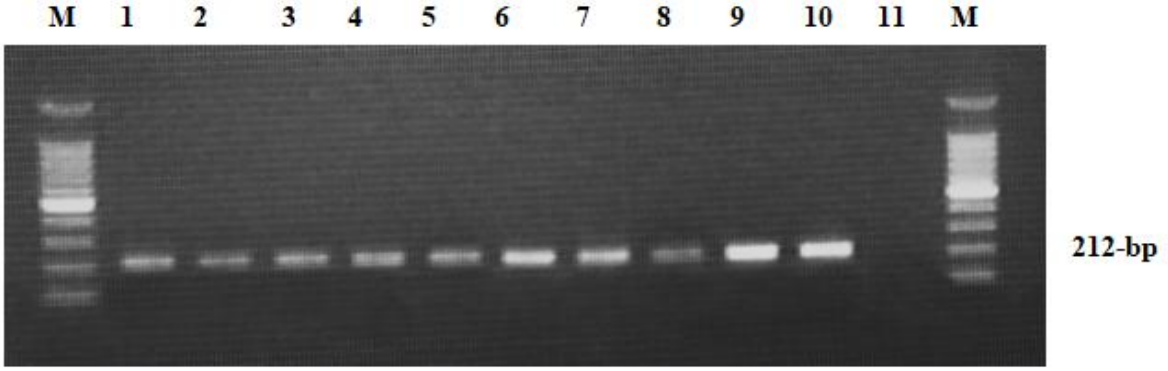

Fig.1: Conventional PCR detection Escherichia coli in chicken, beef and pork meat samples in agarose gel electrophoresis, Lane M: Molecular weight marker, 100 bp, Lane 1-3: E. coli in chicken, Lane 4-6: E. coli in beef, Lane 7-9: E. coli in pork, Lane 10: positive control, Lane 11: Negative control.

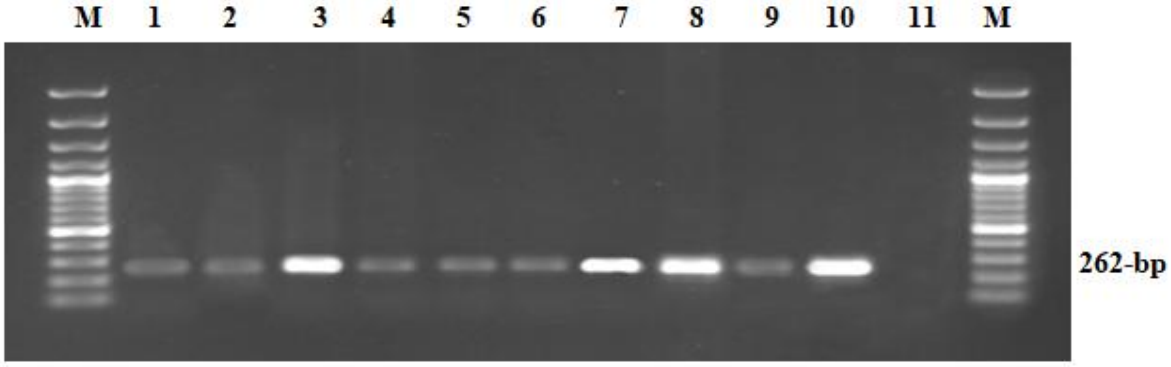

Fig.2:Conventional PCR detection Salmonella spp. in chicken, beef and pork meat samples in agarose gel electrophoresis, Lane M: Molecular weight marker, 100 bp, Lane 1-3: Salmonella spp. in chicken, Lane 4-6: Salmonella spp. in beef, Lane 7-9: Salmonella spp. in pork, Lane 10: positive control, Lane 11: Negative control.

CONCLUSION

Differences in prevalence rates of Salmonella spp. and E. coli in the different types of meats in this work can be attributed to multiple factors, such as geographical and seasonal variation, variations in sampling procedures and animal management practices; Besides, our work is the only one that has focused its study on three types of meat using molecular and conventional methods to the detection in Ecuador.

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Conflict of interest

The authors declare that they have no conflict of interest.

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