

Characterization by PCR of *Escherichia coli* from Beef and Chicken Used in Restaurants in Yaoundé Cameroon

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Abstract

Meat constitutes the main source of protein and occupies an important place in our diet. Indeed, the production of poultry and beef has increased. However, the hygienic quality of meat is not always guaranteed. Microorganisms such as *Escherichia coli* can be found in meat and can cause various infections including diarrhea, dysentery, food poisoning, gastroenteritis or typhoid fever. Thus, the present study was designed to characterize *Escherichia coli* (*E. coli*) from beef and chicken consumed in restaurants in Yaoundé Cameroon. A total of 105 meat samples (60 beef and 45 chickens) were subjected to microbial culture for *E. coli* isolation and further confirmed by Polymerase Chain Reaction (PCR) using primers EC-F and EC-R that are specific to *E. coli* 16S rRNA gene. The supplier source, storage, and transport conditions were taken into consideration during sample analysis and data processing. This study revealed that 77/105 samples (73.33%) were positive for *E. coli* following microbial culture and 35 (33.33%) were positive for *E. coli* following molecular examination. A statistically significant difference was observed

when PCR and microbial culture were used to assess for *E. coli* in beef and a non-statistically significant difference was observed in the case of chicken meat. Also, a statistically significant difference was noticed with the different transport conditions, but this wasn't the case with the supplier source as well as the storage conditions where a non-statistically significant difference was seen. This study revealed that PCR-based methods are fast and reliable in the identification and characterization of *Escherichia coli* in meats (beef and chicken) as well as in assessing the prevalence of pathogenic *E. coli*, in Cameroon.

Keywords

Characterization, Prevalence, *E. coli*, Meat, Polymerase Chain Reaction (PCR)

1. Introduction

Eating is a primary need for everyone. The foods we eat must be healthy and balanced to provide the body with the nutrients necessary for physical and mental well-being. About 25% of the population still suffers from chronic undernourishment in sub-Saharan Africa [1]. Malnutrition is generally rife in poor countries because of nutritional deficiencies. Meat constitutes the main source of protein and occupies an important place in our diet. It has a high protein content and contains all the essential amino acids, iron, zinc and vitamins A, B12, B6, D and E in particular [2]. In addition, meats are rich in lipids and carbohydrates essential for growth and development [3]. Meat can be part of a balanced diet providing important nutrients for health. Fortunately, the production of poultry and beef has increased [4]. World production of bovine meat was estimated at 67.7 million tons and that of poultry meat at 107.0 million tons in 2013 [5]. Meat production in Cameroon reached 344,000 tons in 2016 with around 118,267.2 tons of poultry and over 7.4 million head of cattle [6]. Cattle and poultry meats are marketed in Cameroon in fresh or processed forms (roast, shawarma, beefsteak, kebabs, sausages, etc.). Unfortunately, the hygienic quality of meat is not always guaranteed [7] [8]. Almost one out of ten people in the world fall ill after consuming food contaminated with pathogens and contaminants (bacteria, viruses, parasites, toxins and chemicals) [9]. Foodborne diseases are a global public health problem; apart from the toxi-infections that they can cause very quickly after consumption, they can also cause long-term illnesses, such as cancer, kidney or liver failure and brain or nervous disorders. These diseases can be more serious in children, pregnant women, the elderly or those with a weakened immune system. Microorganisms such as *Escherichia coli*, *Staphylococcus aureus* and *Salmonella* were found in the meat carcasses analyzed which could cause diarrhea, dysentery, various infections, food poisoning, gastroenteritis, or typhoid fever [10].

E. coli is a Gram-negative bacterium belonging to the *Enterobacteriaceae* fam-

ily. They are short rods shaped and motile by means of peritrichous flagella. Moreover, they are facultative anaerobes, non-sporulating and 2 - 4 μm in length and approximately 0.6 μm in diameter, *E. coli* is one of the fecal coliforms normally found in the digestive microflora of humans and many animals such as cattle and poultry. Many *E. coli* species are safe for humans and animals. However, some strains such as *E. coli* O157: H7 are pathogenic for humans. Humans can be healthy carriers of these bacteria, with no visible symptoms. However, ingestion of a few hundred bacteria may be sufficient to reach the infective dose [11]. Their main clinical sign is diarrhea, which is more often hemorrhagic and can be associated with abdominal pain and sometimes vomiting. The incubation phase depends on the virulence of the strain, the dose ingested and the resistance of the patients. Humans can become infected by eating undercooked meats. In effect, *E. coli* is very abundant in the intestinal flora but does not persist for long in the environment and thus, the best indicator of faecal contamination. In the meat industry, the main source of *E. coli* contamination is the intestinal tract of animals. Their presence in meat indicates a technical slaughter defect or cross contamination. It can also be due to contamination from people handling meat [12]. In sub-Saharan Africa, cases of foodborne illness are enormous, but their estimates are largely underestimated by health authorities and their origins are rarely elucidated [13]. In Cameroon, studies carried out so far have shown the presence of pathogenic microorganisms in some meat samples [7] [14] [15]. Thus, the present study was designed to characterize *Escherichia coli* from beef and chicken meat used in restaurants in Yaoundé, Cameroon.

2. Materials and Methods

2.1. Sample Collection

A total of 105 meat samples ($n = 105$) were collected randomly following a convenience sampling method without repetition of meats. Sixty (60) and forty-five (45) samples were from beef and chicken respectively. About 100 g of different bovine and chicken meats were collected under aseptic conditions (NF ISO 18593, 2004). Samples were wrapped in aluminum foil and kept in sterile plastic bags. They were clearly labeled, identified and transported on ice packs (0°C and 4°C), in a cooler (Keep Cold[®]) to the Laboratory of Public Health Research Biotechnology (LAPHER Biotech.) for immediate analysis.

2.2. Microbial Culture

In the laboratory, 10 g of each sample was introduced aseptically into a sterile stomacher bag and macerated in 90 mL of sterile diluent (0.1% peptone and 0.8% sodium chloride from DIFCO LABORATORIES Detroit MI USA). Serial dilutions were carried out by introducing 1 mL of the meat suspension into 9 mL of sterile diluent which was then used for the cultivation of micro-organisms. The various dilutions were done in accordance with the standards of NF EN ISO 6887, 2011. About 0.1 mL of sample from appropriate dilutions were plated on

the following culture media (all from *Biolife*): Total aerobic mesophilic bacteria were seeded on Plate Count Agar (PCA) and incubated at 30°C for 72 h (ISO 4833-1, 2013). Violet Red Bile Glucose Agar (VRBGA) was used to isolate *Enterobacteriaceae* and plates were incubated at 37°C for 24 h (NF ISO 21528-2: 2004). Tryptone Bile X-glucuronide Agar was used for the isolation and enumeration of *E. coli* and incubation was done at 44°C for 24 h (NF ISO 21528-2: 2004).

2.3. DNA Extraction

A loop full of bacteria from the culture plate was placed into a 1.5 mL micro-centrifuge tube and mixed with 200 µL of 5% Chelex-100 resin (Bio-Rad) and 2 µL of Proteinase K (20 mg/ml, NEBiolabs). After incubation at 56°C for an hour and then at 95°C for 10 minutes, the sample was mixed and then centrifuged at 13,000 rpm for 5 minutes to completely separate the layers. The DNA-containing supernatant was used as a template in PCR reactions.

2.4. PCR Confirmation of *E. coli* Isolates

The isolated organisms that were phenotypically identified as *E. coli* were then confirmed by PCR using primers specific to *E. coli* 16S rRNA gene (**Table 1**). PCR was performed following the procedure described by Schippa *et al.*, with slight modification [16]. 25 µL reaction containing nuclease free water, 10× thermopol buffer, 10 mM dNTPs (200 µM of each deoxyribonucleotide), 20 pmol of each primer and 5 U/µL *Taq* polymerase and 3 ng of DNA. After initial incubation at 95°C for 3 min, a 30-cycle amplification protocol was followed at 94°C for 45 s, 58°C for 45 s and 72°C for 60 s, and a final extension step of 72°C for 3 min. Electrophoresis of the PCR products was done using 2% agarose gel. After electrophoresis, the gel was stained for 10 minutes in ethidium bromide for visualization and the expected band size was observed at 585 bp under a UV lamp using a UV transilluminator.

2.5. Data Analysis

Data was entered into excel sheets and statistical analyses were performed using EPI INFO version 7.2.2.16. Using Pearson's Chi-square (χ^2) test difference among the variables was calculated. P values less than 0.05 were considered as significant.

3. Results

3.1. Cultural and PCR Detection of *E. coli*

The isolates upon 24 hours of incubation at 44°C produced blue colored colonies on TBX (Tryptone Bile X-glucuronide) agar. The isolated *E. coli* were subjected to PCR using *E. coli* specific EC-F and EC-R primers. Upon electrophoresis, a 585 bp on agarose gel was characteristic of the presence of *E. coli* by PCR examination (**Figure 1**). A total of 105 meat samples were examined while 77

Table 1. Primers used in this study.

Target gene	Primer name	Sequence (5'-3')	Product size	Reference
16SrRNA	EC-F	GACCTCGGTTTAGTTCACAGA	585 bp	[16]
	EC-R	CACACGCTGACGCTGACCA		

**Figure 1.** Representative photograph of the PCR of *E. coli* targeting 16S rRNA gene. M = 100 bp DNA Ladder, PC = Positive control, 1 - 5 = Test samples, NC = Negative control.

(73.33%) were positive for *E. coli* following microbial culture, and 35 (33.33%) were positive for *E. coli* following molecular examination. the difference was statistically significant ($P < 0.05$). Concerning the type of meat samples, PCR examination had detected *E. coli* less than microbial culture examination. In beef samples, there was a statistically significant difference between phenotypic and genotypic identifications. However, in chicken samples, there was no statistically significant difference between both methods (**Table 2**).

3.2. Prevalence of *E. coli* under Different Handling Conditions

The study was also focused on the prevalence of *E. coli* in different suppliers' sources, storage conditions and transport conditions. According to this study, the prevalence of *E. coli* was low in all parameters. The difference was statistically significant among different transport conditions but was not statistically significant among different suppliers' sources and storage conditions (**Table 3**).

4. Discussion

Escherichia coli is considered as the most important serotype of the Enterohemorrhagic *E. coli* and plays an important role in the incidence of ulcerative colitis, thrombotic thrombocytopenic purpura, and hemolytic uremic syndrome [17]. Hemolytic uremic syndrome occurs in 2% - 7% of patients and results in death in 3% - 5% of cases [18]. Although it has been proved that this bacterium is found in all geographical areas and can be isolated from different kinds of foods, its occurrence varies in different regions depending on the type of food, the sampling season and the methods of isolation [19] [20].

This study revealed the presence of *E. coli* in 73.33% following microbial culture and 33.33% following molecular examination. The results of PCR procedure

Table 2. Culture and PCR detection of *E. coli*.

Type of sample meat	Cultural examination			PCR examination			P value (χ^2 test)*
	Positive	Negative	Total	Positive	Negative	Total	
Beef	46 (76.67%)	14 (23.33%)	60	25 (41.67%)	35 (58.33%)	60	0.048*
Chicken	31 (68.89%)	14 (31.11%)	45	10 (22.22%)	35 (77.78%)	45	0.414
Total	77	28	105	35	70	105	0.043*

*Statistically significant.

Table 3. Prevalence of *E. coli* in different parameters.

Parameters	Positive	Negative	Total	P value (χ^2 test)*
Supply source	Slaughterhouse	14 (66.67%)	7 (33.33%)	21
	Farm	4 (19.05%)	17 (80.95%)	21
	Traditional butchery	14 (33.33%)	28 (66.67%)	42
	Modern butchery	4 (19.05%)	17 (80.95%)	21
Storage condition	Freezer	21 (33.33%)	42 (66.67%)	63
	Cold room	21 (50%)	21 (50%)	42
Transport condition	Cooler	11 (31.43%)	24 (68.57%)	35
	Simple bag	10 (23.81%)	32 (76.19%)	42
	Refrigerated car	14 (50%)	14 (50%)	28

*Statistically significant.

demonstrated that out of the 105 samples, 35 cases were identified as *E. coli*. These results confirmed the importance of molecular methods in detecting this pathogen. This method not only can save time and labour but also is very cost-effective. Therefore, it is suggested as a superior procedure for isolating *E. coli* from foods. In this regard, previous research from Iran declared that the PCR procedure is a fast method to detect the *E. coli* in the pharmaceutical, food, and water industries [21]. In another study in 2009 in Iran, researchers have proved that PCR-based methods are fast and reliable methods to detect and identify as few as 100 cells of *E. coli*, *Vibrio cholerae*, and *Salmonella typhimurium* [22].

This study revealed the presence of 33.33% *E. coli* in meat (beef and chicken), as well as the prevalence of *E. coli* in different parameters. According to this study prevalence of *E. coli* was low in supplier source, storage and transport conditions. Similar studies around the world have also reported a low prevalence of *E. coli* in meat. Akomoneh *et al.* [23] reported a prevalence rate of 10.9% in Buea Cameroon, Luga *et al.*, [24] reported a prevalence rate of 9% in neighbouring Nigeria and Hiko *et al.*, [25] and Bekele *et al.* [26] respectively reported 8% and 10.2% in Ethiopia. Callaway *et al.* [27] reported a prevalence rate of 11.3%, in the United States, Syngge *et al.* [28] and Omisakin *et al.* [29] reported

prevalence rates of 8.6% and 7.5% respectively in the United Kingdom while Hashemi *et al.* [30] had a prevalence rate of 8.3% in Iran. According to Mouafo *et al.* [31], the prevalence of *E. coli* in meat is 60% in Ngaoundéré, Cameroon and Kimassoum *et al.* [32] showed that the prevalence of *E. coli* in meat was 55% in Yaoundé, Cameroon and 70% in N'Djamena, Chad. These differences might be due to the differences in the methodology employed in these studies. Mouafo *et al.* [31] and Kimassoum *et al.* [32] described their findings based on microbial culture and biochemical analysis. However, in this study, in addition to traditional techniques, PCR was employed for the confirmatory identification of *E. coli* from sample meat.

5. Conclusion

The study revealed that PCR-based methods are fast and reliable methods to detect and identify as few as 100 cells of pathogenic *E. coli*, as well as in the determination of the prevalence of pathogenic *E. coli*, in Cameroon. Considering the circulation of this pathogen and human risks, we recommend that restaurant and abattoir workers should be trained on the basic concepts and requirements of food and personal hygiene as well as those aspects particular to the specific food-processing operations including waste disposal.

Authors' Contributions

Wilfred Fon Mbacham, Ahmadou Hamadjam Alkaïssou, Jean Paul Kengne Chedjou, Justin Ledoux Tanke Fanjip, and Palmer Masumbe Netongo contributed to the design of the study. Jean Paul Kengne Chedjou, Palmer Masumbe Netongo, and Justin Ledoux Tanke Fanjip coordinated the study. Justin Ledoux Tanke Fanjip and Ahmadou Hamadjam Alkaïssou supervised the sample collection. Justin Ledoux Tanke Fanjip, Carolle Eyébé Nsa'amang, and Ngum Lesley Ngum performed cultural examination. Jean Paul Kengne Chedjou, Justin Ledoux Tanke Fanjip, Aristid Ekollo, and Mbu'u Mbanwi Cyrille performed the molecular analysis. Palmer Masumbe Netongo and Serge Eyébé performed data analysis. Jean Paul Kengne Chedjou, Justin Ledoux Tanke Fanjip, Serge Eyébé, and Ngum Lesley Ngum wrote up the manuscript. All authors contributed in the revision of the manuscript and approved the final version of the manuscript prior to submission.

Conflicts of Interest

Authors declare no conflict of interest.

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