ERIC-PCR analysis of the genotypic diversity of poultry and human Salmonella strains from N’djamena and Doba towns in Chad

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ABSTRACT
Salmonella is one of the leading causes of food contamination in non-developed and industrialized countries. They represent a significant burden for the public health and a considerable cost for the society. The poultry sector is considered as one of the major sources of human contamination. The objective of this study was to evaluate the genotypic links of Salmonella strains within the various identified serotypes, in order to specify the variety of clones circulating within the poultry sector and to determine the zoonotic character of salmonellosis. Salmonella strains used were screened from 1090 samples taken from 10 broiler farms in N’Djamen and 16 traditional poultry farms in Doba. These strains were subjected to molecular characterization through ERIC-PCR technic that confirmed their belonging to the genus Salmonella, with diversity both within and between different serotypes. The identical genetic profiles of some serotypes, has confirmed the diffusion and persistence of the same clones throughout the pathway, but also the polymorphism of other serotypes, indicating the diversity of potential reservoirs of non-typhoid Salmonella. The comparison of human and poultry ERIC-PCR profiles revealed indirect evidence of Salmonella serovar contaminants from human origin in poultry farms of N’Djamen and Doba towns. The evidence of the Salmonella and E. coli germs in both their phenotypic and genetic diversities constitute a serious constraint to be integrated in the control strategies of human and animal diseases caused by these enterobacteria.

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1. Introduction:
The genus Salmonella has continued to be of considerable importance in the veterinary and medical fields [1]. As a result, non-typhoidal Salmonella infections constitute a public health problem [2]. The poultry sector is considered as one of the major sources of human contamination [3], via food consumed raw or undercooked: poultry meat, eggs and egg-based preparations, egg products, cold meats [4-5-6-7-8]. In addition, the emergence of resistant strains of Salmonella is gradually increasing in Chad as in other countries [9-10]. On the other hand, prevention against salmonellosis involves surveillance at local (industry, hospital) and national (reference center) levels, based on discriminant typing of strains [11]. The identification of Salmonella at the species level remains insufficient. Thus, an arsenal of biological techniques based on nucleotide sequence variations have recently been developed to study the genetic diversity of these bacteria [12-13].

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include a set of typing techniques more discriminating between the strains of *S. enterica*; ribotyping [14], ERIC-PCR reaction [15], Amplifying Fragments Length Polymorphism or AFLP [16], Random Amplifying Polymorphism or RAPD [13], Pulsed Field Gel Electrophoresis or PFGE [17], Polymerase chain reactions-Restriction Fragments Length Polymorphism (PCR-RFLP) [18], typing by determination of the nucleotide sequence of several genes coding for essential functions to bacteria through Multi-Locus Sequence Typing (MLST) [19]. Since 2003, genotyping method by Multi-Locus Variable Number of Tandem Repeat Analysis (MLVA) has shown considerable higher reproducibility and discrimination ability than the PFGE [20]. Considering such an evolution, which reflects a multitude of chromosomal and extra-chromosomal genetic supports, an epidemiological survey has become essential in order to visualize and control the variability with different genotypes. Hence, different systems of survey generally organized in national and international networks have been installed, which aim at studying the distribution of serovars, monitoring the development of antibiotic resistance at different levels (from animals to humans) in order to better adapt the control and prevention measures [21]. In our intention of participating to the evaluation of the *Salmonella* diversity in the poultry sector in N’Djamena and Doba, and considering that no single research has been conducted on consumed food that could vehiculate human salmonellosis in Chad, this study was undertaken to assess the genotypic links between different *Salmonella* serotypes identified by ERIC-PCR, such as to specify the circulating clones and prove the zoonotic character of salmonellosis.

2. Material and methods:

2.1. Sampling, Isolation and confirmation of bacteria strains:
Salmonella strains used were isolated from 1090 samples taken from 10 broiler farms in N’Djamena and 16 traditional poultry farms in Doba. The isolation and confirmation of strains were carried out within the general bacteriology laboratory at the Research Institute of Breeding for Development (IRED) of N’Djamena in Chad according to the reference method NF EN ISO 6579: 2002 [22].

Pre-enriched samples to 1/10 with peptinated water buffer and homogenized using a vortex for 2 min were left for revivification at room temperature for 30 min, then incubated at 37 °C for 6-20 hours. Then 0.1 ml of the pre-enriched sample was used to inoculate 10 ml of Rappaport Vassiliadis Soja medium (RVS), of which 1 ml served to inoculate 10 ml of Mueller-Kaufmann Tetrathionate medium (MKTTn). Subsequently, the RVS and MKTTn media were respectively incubated at 42 °C and at 37 °C for 24 hours. Finally, the selective Hektoën gel media and Xylose Lysine Désoxycholate (XLD) from the enriched products were inoculated and incubated for 24 hours at 37 °C. On average, 05 characteristic colonies were sampled and transferred onto Hektoën agar for the first purification, followed 24 hours later by the second purification [22]. The biochemical identification of *Salmonella* strains was assessed by culturing the inoculum on the API® 20E gallery (Bio-Merieux). The principle is based on the inoculation of microtubules with a suspension, which rehydrates the media. The incubation is carried out at 37 °C. for 24 hours in a bacteriological oven during which the biochemical reactions of decarboxylation, fermentation and deamination take place, and result in spontaneous colored products revealed by the addition of reagents [23]. *Salmonella* identification was performed using API® 20E catalog, according to the instructions of the French Society of Microbiology (SFM).

2.2. Polymerization chain reaction (PCR) of *Salmonella* strains DNA material:

2.2.1. Extraction of total genomic DNA:
The purified bacterial cultures were incubated at 37 °C for 24 hours, after which, 05 well isolated colonies from the bacterial culture were resuspended overnight in 5 ml of Brain Heart Broth culture at 37 °C. The next day, 1.5 ml of the broth were introduced into Eppendorf tubes before centrifugation at 12,000 rpm for 10 min. Subsequently, the supernatant was carefully removed. The precipitate thus obtained was re-suspended in 100 μl phosphate buffered saline (PBS), and re-centrifuged at 12,000 rpm for 10 min to wash the cells of the medium. The supernatant was removed again, then the precipitate was dissolved in 100 μl of distilled water by boiling for 10 min, re-centrifuged again at 12,000 rpm for 10 min to separate DNA from other bacteria debris. The supernatant was finally aliquoted into another Eppendorf tube, then stored at -20 °C to serve as DNA for the amplification reactions.

2.2.2. Genomic DNA amplification of by ERIC-PCR:
The technique is based on the Polymerase Chain Reaction (PCR), which amplifies DNA sequences *in vitro* by enzymatic synthesis. The technique is automated through a thermostable enzyme (Taq DNA polymerase) isolated from a thermophilic bacterium (*Thermobacterium aquaticus*), adapted to life in hot springs [24], in the presence of specific primers and nucleotides (dNTP). Amplified PCR products of *Salmonella* strains are analyzed by
ERIC-PCR. In principle, the Enterobacterial Repetitive Intergenic Consensus (ERIC)-Polymerase Chain reactions (PCR) products indicate the existence of a diversity within the genus or bacterial clones. In this PCR process, the genomic DNA regions to be amplified correspond to highly repeated and conserved sequences in the bacterial genome. It accounts for the dispersion of characteristic DNA sequences of each bacteria strain on one hand, and on the other, proposes a more detailed analysis of the bacteria genome belonging to close related phylogenetically or distant groups. The ERIC unit sequences, also known as repeating intergenic units are present in multiple copies within the genome of *Salmonella*, *E. coli* and other enterobacteria. These elements of 126 bp long are very well preserved at the nucleotide level. The position of these elements in the genome of enterobacteria varies according to species and can be used as a genetic marker to characterize the isolates bacteria species [15]. Poultry salmonella strains are examined for specific genes encoding the ERIC sequences using specifically the ERIC-1R and ERIC-2 oligonucleotide primer sequences (table 1). The lyophilized oligonucleotides supplied by Eurofins Genomic, Anzingerstabe 7a, D-85560 Ebersberg were diluted according to the synthesis report of oligonucleotides supplied by the manufacturer. The diluted solutions were then divided into aliquots and then stored at -20 °C for the amplification step.

Table 1. Primers sequences.

<table>
<thead>
<tr>
<th>Primers</th>
<th>Nucleotide sequences (5' → 3')</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERIC 1R</td>
<td>ATG-TAA-GCT-CCT-GGG-GAT-TCA-C</td>
<td>[25]</td>
</tr>
<tr>
<td>ERIC 2</td>
<td>AAG-TAA-GTG-ACT-GGG-GTG-AGC-G</td>
<td>[25]</td>
</tr>
</tbody>
</table>

2.2.3. Preparation of the reaction mixture:
The final concentration volumes of each reagent to be used were determined based on the initial concentration of the used solution. All the PCR reaction components were thawed on ice. The reaction mixture, commonly called mix was calculated for all of the isolates to be tested and prepared by adding reagents one after the other: ultra-pure water; dNTPs; pair of sense/antisense primers; PCR buffer and the enzyme Taq polymerase, which is introduced last into the reaction mix. The reaction mix was then redistributed between the negative and positive controls and the samples. The DNA to be amplified was subsequently added to the reaction mixture. The PCR reactions were carried out in 0.1 ml microtubes, for a reaction volume of 25 μl, the composition of which is given in table 2.

Table 2. Composition of the ERIC-PCR reaction mix.

<table>
<thead>
<tr>
<th>Components</th>
<th>Reaction volume (μL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O</td>
<td>18.8</td>
</tr>
<tr>
<td>Tampon de PCR 10X (MgCl2)</td>
<td>2.5</td>
</tr>
<tr>
<td>dNTP (10 mM)</td>
<td>0.5</td>
</tr>
<tr>
<td>Amorce ERIC 1R (12.5 pmol/μL)</td>
<td>0.25 (50 μM)</td>
</tr>
<tr>
<td>Amorce ERIC 2 (12.5 pmol/μL)</td>
<td>0.25 (50 μM)</td>
</tr>
<tr>
<td>Taq DNA polymerase (5 U/μL⁻¹)</td>
<td>0.2</td>
</tr>
<tr>
<td>Extraits d'ADN</td>
<td>2.5</td>
</tr>
</tbody>
</table>

2.2.4. Amplification conditions:
The different steps of DNA amplification were carried out in successive cycles in a manually programmed Thermocycler (Mastercycler Personal), and within AG 22331 Eppendorf type. The variable parameters were the time, the temperature of each amplification step, as well as the number of cycles. The amplification respected the following steps: pre-denaturation at 94 °C for 3 min; hybridization at 48 °C for 1 min; elongation at 72 °C for 4 min for the first cycle; followed by denaturation at 94 °C for 1 min; hybridization at 51 °C for 1 min and elongation at 72 °C for 4 min for the next 35 cycles, with the last cycle comprising a denaturation step at 94 °C for 1 min, hybridization at 51 °C for 1 min and a final elongation at 72 °C for 10 min [25].

2.2.5. Agarose gel electrophoresis separation of ERIC PCR products:
The migration gel was a 2% agarose gel (mass/volume), composing 3 g agarose dissolved in 150 ml of TBE solution, 1 time concentrated (1×). The mixture was heated to boiling in the microwave for about 3 minutes to obtain a clear, homogeneous phase. Then in the still liquid cooled agarose, 15 μl of Ethidium Bromide was added. The solution obtained was poured into a support, on which was fixed a comb forming-wells necessary for the
deposition of the amplified products. After approximately 30 minutes of polymerization, the gel was placed in the electrophoresis tank, containing the TBE buffer. With the comb removed, each well was loaded with 12 μl of PCR-products, previously mixed with 2.5 μl of loading buffer (0.2g Bromophenol Blue 3 mmol.L⁻¹, 5.1g sucrose 1.5 mol.L⁻¹, 0.01g Tris-HCl 10 mmol.L⁻¹ [pH 8]). The first well was generally loaded with 12 μl of 100 bp DNA molecular weight marker, mixed with the loading buffer comprising several DNA fragments of known size and serving as a benchmark scale. An electrical current of 120 volts was applied to the tank for 45 minutes, with the wells positioned on the cathode side. After migration, the amplified products were visualized by the fluorescence of Ethidium Bromide under ultraviolet light at 254 nm using a polaroid device for image capture.

2.2.6. Definition of controls and human strains:
For all the amplification reactions, the Negative Control (NC) was composed of the reaction mixture and ultrapure water, whereas the Positive Control (PC) and the rest of the human strains (H1, H2, H3, H4, H5) were graciously provided by the Bacteriology Laboratory of the NDjamena General Reference Hospital where they were serotyped and confirmed by the Vitek® 2 Compact 15: TP: Salmonella Typhi; H1: Salmonella Hadar; H2: Salmonella Limete; H3: Salmonella Anatum; H4: Salmonella Paratyphi A; H5: Salmonella Typhi.

3. Results and discussions:
The forty strains of poultry Salmonella isolated, as well as the five human strains collected were characterized by ERIC-PCR. For each strain, the experiment was repeated thrice. For each serotype groups, an enterobacteria Repetitive Intergenic Consensus Polymerase Chain Reaction (ERIC-PCR) was used as a genetic marker, in order to compare the phylogenetic profiles and the cloning links of our strains with each other. In each group of common strains, a representative isolate was then chosen and characterized again, one next to the other on the same gel.

The size of the amplified DNA fragments on the agarose gel was evaluated by co-migration of the DNA fragments with the marker 100, harboring bands between 100 and 3000 bp. Generated DNA fragments from different isolates revealed on agarose gel were composed of 3 to 10 bands with sizes varying between 147 bp and 3 kb. The poultry strains isolated from meat farms were numbered from 1 to 17 (Figure 1), while the poultry strains isolated from traditional farms were identified from A to Q (Figure 2). Human strains, on the other hand, were identified by the letter H, followed by an index of number from 1 to 5 (Figure 3).

For each ERIC profile obtained, we identified the position of bands referring to the molecular weight marker 100. This enabled us to molecularly group strains with identical ERIC profiles. We thus highlighted twenty-five different ERIC-PCR profiles of poultry and human Salmonella, numbered from I to XXV (Figure 1, 2 and 3).

3.1. ERIC-PCR profiles of poultry and human Salmonella strains:
Poultry Salmonella strains isolated from meat farms (figure 1) indicate 11 different ERIC-PCR profiles. Within the four strains of S. Mbandaka, 3 different ERIC-PCR profiles were observed, namely IV (approximately 7 bands), V (approximately 6 bands) common to two strains, and VI (approximately 6 bands) common to two other strains, with profile V identical to profiles III of S. Limete, VIII of S. Anatum and X of S. Idikan. The seven strains of S. Idikan also presented two different ERIC-PCR profiles, including X (approximately 6 bands) common to five strains, and XI (approximately 5 bands) shared by two other strains in common. Moreover, the three strains of S. Anatum each showed a different ERIC-PCR profile, respectively VII, VIII and IX with nearly 6 bands each. Concerning the poultry Salmonella strains from traditional farms, 13 ERIC-PCR profiles were identified (Figure 2), including 2 different profiles, XII (approximately 7 bands) appearing in two strains, XIII (approximately 5 bands) in one strain of the Derby serovar. The two strains each of S. Virchow, S. Anatum, S. Limete showed different ERIC-PCR profiles, respectively XVI (approximately 6 bands) and XVII (approximately 5 bands), IX (approximately 6 bands) and XXI (about 3 bands), XVIII (about 6 bands) and XIX (about 9 bands). In the reverse, a strain of the S. Limete serotype showed an ERIC-PCR profile (XVIII) similar to profile XX of the S. Enteritidis serotype. As for human strains, each serotype presented a different ERIC-PCR profile (Figure 3), comprising profile XXII (approximately 5 bands) that was found in the serovar Limete, profile IX (approximately 6 bands) in the serovar Anatum and profiles XXIII, XXIV and XXV (approximately 3 bands each) in the serovars Hadar, Paratyphi A and Thyphi respectively.
Figure 1. ERIC-PCR profiles of some *Salmonella* from poultry farms from N’Djamena.

M: Molecular weight marker 100 pb; TN: Negative control; TP: Positive control; 1: *Salmonella* Infantis; 2: *Salmonella* Paratyphi A; 3: *Salmonella* Limete; 4, 5, 6, 7: *Salmonella* Mbandaka; 8, 9, 10: *Salmonella* Anatum; 11, 12, 13, 14, 15, 16, 17: *Salmonella* Idikan.

Figure 2. ERIC-PCR profiles of some *Salmonella* from poultry farms from

M: Molecular weight marker 100 pb; TN: Negative control; TP: Positive control; 1: *Salmonella* Infantis; 2: *Salmonella* Paratyphi A; 3: *Salmonella* Limete; 4, 5, 6, 7: *Salmonella* Mbandaka; 8, 9, 10: *Salmonella* Anatum; 11, 12, 13, 14, 15, 16, 17: *Salmonella* Idikan.

Figure 3. ERIC-PCR profiles of some human *Salmonella*

3.2. Comparison of ERIC-PCR profiles of common Salmonella serotypes:
In order to assess the genetic links between Salmonella from broiler and traditional poultry farms, and their contributions to human salmonellosis, an analysis was carried out during which ERIC-PCR profiles of some Salmonella belonging to the same serotypes, and isolated from traditional poultry farms on the one hand (Figure 4), and from human (Figures 5) were compared. ERIC-PCR profiles of common Salmonella serotypes of poultry origin from meat and traditional farms were observed. Four common Salmonella serotypes encountered in poultry and traditional farms showed 8 different ERIC-PCR profiles (Figure 4). One S. Infantis strain from poultry farms and two other from traditional farms indicated an identical ERIC-PCR profile, whereas one S. Limete strains from poultry farm and two from traditional farms revealed different profiles. Furthermore, 3 different ERIC-PCR profile of S. Anatum serotypes from poultry farms, and 2 from the traditional farms were obtained, of which 2 were identical for each farm, whereas the 3rd was different from those of the traditional farm. The 5 S. Idikan serotypes from poultry farm and one from the traditional farm revealed 2 undistinguishables ERIC-PCR profiles, one of which regrouped 3 of the poultry farm serotypes and the unique traditional farm serotype, while the other profile assembled the two serotypes of the poultry farm.

![Figure 4](image)

**Figure 4.** Differences in the ERIC-PCR profiles of Salmonella from poultry and traditional farms.
M: Molecular weight marker 100 pb; TN: Negative control; TP: Positive control; 1, D, E: Salmonella Infantis; 3, L, M: Salmonella Limete; 8, 9, 10, O, P: Salmonella Anatum; 11, 12, 16, 13, 17, Q: Salmonella Idikan.

3.3. Comparison of ERIC-PCR profiles serotypes of Salmonella from poultry and human origins
Among all the studied serotypes, only the human S. Anatum and poultry strains showed an undistinguishable ERIC-PCR profile (Figure 5), all the other have indicated different profiles.

![Figure 5](image)

**Figure 5.** Variation in the ERIC-PCR profiles of Salmonella from poultry farms and of human origin.
M: Molecular weight marker 100 pb; TN: Negative control; TP: Positive control; H1: Salmonella Hadar; H2, M: Salmonella Limete; H3, O, I0: Salmonella Anatum; D: Salmonella Infantis; H4: Salmonella Paratyphi; B: 2: Salmonella Paratyphi A; H5: Salmonella Typhi; B: Salmonella Derby; J: Salmonella Virchow; N: Salmonella Enteritidis; 6, S: Salmonella Mbandaka; 16, 12: Salmonella Idikan

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In the present study, ERIC-PCR gene amplification technic has shown to be quite efficient for an initial classification of clones, in particular for certain strains such as S. Infantis and S. Anatum. In fact, it is a sensitive technic that has the advantage of being simple, quick and less expensive compared to other genotypic technics. Kumao et al. (2002) reported that the ERIC-PCR technic could help for the intraserotypic typing of Salmonella strains and be recommended as a method of choice for the detection of epidemic diseases [26]. Among all the studied serotypes, only S. Infantis presented an ERIC-PCR profile (I) not distinguishable from all the other serovar strains. On the other hand, S. Mbandaka presented an ERIC profile (V), which was similar to the ERIC profiles (VIII) of S. Anatum, (X) of S. Idikan and (III) of S. Limete, which also presented an ERIC profile (XIX) identical to that of S. Enteritidis (XXI). ERIC-PCR may not be very discriminating against the above strains, suggesting a limited interest in using this approach for the epidemiological study on Salmonella. This result contradicts those of Van Lith and Aarts (1994), but is similar to that of Millemann et al. (1996), who despite preliminary attempts to optimize the reaction conditions, have not obtained reproducible profiles among 56 S. Typhimurium studied strains, but only one among 29 S. Enteritidis serotypes.

The comparison of the genotypic profiles of the isolated strains based on their serotypes and origins has revealed that 3 S. Infantis isolates were encountered in three different circumstances, of which 2 were from two traditional farms during two different collection campaigns, while the other that shared an identical profile was found in a broiler farm. This could mean that these isolates are clones of the same origin and circulating between meats sampled from traditional farms in the cities of N'Djamena and Doba. This result clearly confirms the poultry reservoir of S. Infantis serotype and suggests the persistence of clones in the traditional farms at Doba in the two consecutive collection campaigns. Indeed, Mammina et al. (2003) reported that clonal dissemination of some predominant genotypes may be more widespread than is commonly thought, and that national clonal dissemination through poultry is possible. Four S. Limete isolates from two traditional chicken (XVIII, XIX), meat (III) and human (XXII) farms shared four different ERIC-PCR profiles, demonstrating a high discrimination power of ERIC-PCR method, thus providing a proof that these isolates are not clones of S. Limete, and hence, emphasizing the various origins of the sources of contamination. Undoubtedly, the present study has presented an identical profile between S. Limete (III) and S. Mbandaka (V), and between S. Limete (XVIII) and S. Enteritidis (XX). The ERIC technic seems to discriminate these serotypes unlike other studies approaches, which have reported the clonal nature and the limits of the genotypic diversity of S. Enteritidis strains [27].

Previously, Thang et al. (1995) showed that 29 of the 32 strains of S. Enteritidis isolates from sporadic infections had the same PFGE profile, but without any epidemiological link. According to Mammina et al. (2003), the clonal spread of certain predominant serotypes such as S. Enteritidis through poultry products may be greater, contrary to result of the present study, in which isolates belonging to this serotype were little encountered.

Two poultry S. Anatum strains of the serovar Anatum from traditional and poultry farms and the only human strain identified had the same ERIC-PCR profile (IX). It is likely that they are strains of the same clone, circulating in these Chadian regions and contaminating different sectors. The genetic similarity of this serotype between the different isolates from poultry and human origins must be interpreted with care, because we only evidenced one human strain in this comparison, and therefore, it would be difficult to draw conclusions about the possible epidemiological link. Furthermore, this serovar may not be common only in all poultry farms, since its isolation may also come from water and food samples. This could suggest contamination of the environment and particularly of other animals species that have free access to livestock (cattle, sheep, and rats), or even human contamination. Two other poultry strains of the same serovar, from traditional and poultry farms presented a second undistinguishable profile, as well as a different profile for a third strain from traditional farms. It can therefore be assumed that these isolates are not clones and that the sources of contamination can be of multiple origins. As for S. Idikan serotype, two different ERIC-PCR profiles (X and XI), with identical profile X for three of the five strains from meat farms were obtained, with the only isolate from traditional farms and a similar profile XI for the other two isolates from meat farms. These profiles suggest epidemiological links between the strains isolated in meat farms and those found in traditional farms, even if some strains had different profiles. These mixed profiles demonstrate the interest of associating the ERIC-PCR with other characterization technics and their complementarity to precisely trace the dissemination or the persistence of strains in farms. The results from the ERIC-PCR method provide evidence of the existence of obvious molecular diversity among Salmonella serotypes isolated from this study. Our results are in agreement with several studies [28-10] carried out on Salmonella strains involving ERIC sequences. They were described for the first time as repetitive intergenic

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units in *E. coli*, *S. Typhimurium* and certain enterobacteria. The ERIC sequence was an imperfect palindrome of 126 bp, highly conserved in these species [15-29]. This method has therefore been used in the past with reliability to discriminate *Salmonella* strains, because isolates belonging to the same strain often show identical profiles [30]. These authors already suggested at that time, the use of the primers of ERIC-1R and ERIC-2 for the discrimination between *Salmonella* serotypes. When testing the same ERIC primers, Millemann et al. (1996) concluded that this method was unable to discriminate between certain *S. Enteritidis* and *S. Typhimurium* isolates. Burr et al. (1998) also reported that the ERIC-PCR was not very practical for the identification of serotypes, but, more recently, Lim et al. (2005), Rasschaert et al. (2005) have demonstrated that it was possible to use ERIC-PCR to differentiate between *Salmonella* serotypes. Obviously, ERIC-PCR method, combined with other molecular typing methods, can not only be used as an appropriate method for grouping and reducing the number of *Salmonella* strains to be serotyped during certain epidemiological investigations [31], but can also be effectively used to identify and trace sources of pathogenic *Salmonella* in food. According to Li et al. (2011), the ERIC-PCR method can also be used to establish the molecular fingerprints of *Salmonella* strains, to analyze their genetic similarity in order to provide molecular epidemiological evidence to prevent and control food-borne *Salmonella*. Grouping isolates or establishing the relationships between isolates is mainly based on the comparison of the profiles obtained based on their phenotypic or molecular identity. The molecular characterization of our isolates from poultry farms and human, therefore, provides descriptive information on the genotypic diversity of the genus *Salmonella*, through the various isolated serotypes, thus enabling suggestion of the hypotheses on contamination and fluxes between trains in the study areas. This molecular characterization method can not only elucidate the source of contamination or the vehicle responsible for the epidemic, but can also enable the following up of the spread and dynamics of a bacteria clone within animal farms and in the processing chain [32-33].

4. Conclusion:
As the outcome of this study, 25 amplified genomic DNA profiles of *Salmonella* were obtained by PCR-ERIC, of which 11 were poultry *Salmonella* strains from meat farms, 13 of poultry *Salmonella* strains from traditional farms, while 5 represented human *Salmonella* strains. These results have confirmed the belonging of different strains to the genus *Salmonella*, attesting to a significant extent the discriminating characterization power of the ERIC-PCR method, as well as the genetic diversity of the studied strains. However, the identical genetic profiles of some *Salmonella* serotypes confirms the diffusion and persistence of the same clones through the poultry sector. Furthermore, the clonal links between certain serotypes suggests the diffusion of some *Salmonella* strains between poultry meats and human as consumers.

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References:

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