

DETECTION AND TYPING OF *SALMONELLA* SP. OF TRADITIONAL CHICKENS AT THE POULTRY SLAUGHTERHOUSE OF THE CENTRAL MARKET OF GAROUA IN BÉNOUÉ, NORTHERN REGION OF CAMEROONBodering Alain^{*1}, Mayore Atéba Djibrine², Gomoung Doloum³, Ngandolo Bongo Nare⁴, Ndoutamia Guelmbaye¹, Ngakou Albert⁵

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ABSTRACT

In order to assess the prevalence of *Salmonella* contaminations in the city of Garoua, this study investigated the research and serotyping of *Salmonella* strains within the traditional chicken industry at the poultry slaughterhouse of the central market of Garoua in Bénoué, Northern Region of Cameroon. Still very present throughout Cameroon, this structure can represent a source of contamination as well as a zoonotic hazard. For this purpose, the study was carried out on 400 chicken carcasses and analyses were performed on cloacal swabs, by bacteriological culture and confirmed by biochemical analyses and PCR to detect the *invA* gene specific to *Salmonella* sp. Thus, 05 strains of *Salmonella* were isolated, a contamination prevalence of 1.3% observed. Serotyping of the isolates resulted in the identification of five different serovars, including Hadar, Idikan, Mbandaka, Infantis and Anatum (n=1, 20% each). These serotypes developed resistance to tetracycline, amikacin, nalidixic acid, Kanamycin and cephalothin. Antibiotics available on the market at low cost and used without precise diagnosis and in insufficient or overdosed doses in the veterinary environment, both in food and for the treatment of bacterial infections. This study has an important impact because it showed for the first time the presence of *Salmonella* in the traditional chicken industry in the Northern Region of Cameroon. The project also highlighted the role that this type of structure can have in the maintenance and circulation of pathogens within animal and human populations. Indeed, in view of the weak biosecurity measures applied, family farming systems represent an important risk factor for the spread of *Salmonella* in both animals and humans, and could be resistant to certain antibiotics.

Keywords: Isolation, typing, *Salmonella* sp., PCR, traditional chicken, antibiotic

INTRODUCTION

The genus *Salmonella* contains more than 2,000 different serotypes that can be found in soil, water and several residues (Chambers *et al.*, 1998). The *Salmonella* genus contains several serotypes that are pathogenic to humans and animals, with domestic and wild animals as reservoirs (Rostagno *et al.*, 2006). Transmission of *Salmonella* infections is mainly through the ingestion of contaminated water or food. Salmonellosis is one of the most frequently reported foodborne diseases worldwide (WHO, 2000). There are many possible routes of transmission for these microorganisms, but commercially raised chickens have been identified as one of the most important (WHO, 2000). With this in mind, FAO and WHO have conducted risk assessments of *Salmonella* in chickens (Salm-Surv, 2005). These assessments provided an overview of the available knowledge on *Salmonella*.

Although there are few specific data on the burden of foodborne disease associated with *Salmonella* in chicken, it is considered to be significant, although the risk varies depending on the control measures and methods implemented throughout the chain from primary production to final preparation for consumption (FAO/WHO, 2009). The presence of these microorganisms in chicken also affects trade, with profound economic impacts. The human health impact and associated costs, trade disruption and the cost of implementing effective control measures led the Codex Alimentarius Commission to agree in 2007 that the development of guidelines for the control of *Salmonella* in chickens was a priority.

The increase and accumulation of antibiotic resistance is another aspect of the public health problem of salmonellosis, as it is currently accepted in both developed and developing countries that some of the multi-resistant *Salmonella* found in humans are of animal origin and acquired their resistance genes in farms before transmitting them to humans through food (Ungemach *et al.*, 2006).

Therefore, the present study was undertaken with a view to investigating and confirming the existence of *Salmonella* sp. within the traditional chicken industry at the poultry slaughterhouse of the central market of Garoua in Bénoué, Northern Region of Cameroon.

MATERIALS AND METHODS

Biological material

The chicken carcasses used for the samples all come from the poultry slaughterhouse in the central market of Garoua in the Northern Region of Cameroon. Slaughter here is based on seasonal markets in neighboring villages: Ngong and Gashiga on Monday, Babla and Ouro Labo on Tuesday, Bé and Bamé on Wednesday, Adoumri and Baïla on Thursday, Guide on Friday, Oulaïbi on Saturday, Pitoa on Sunday.

Sampling

A total of 400 cloacal swab samples were collected from 400 traditional chicken carcasses at a rate of 10 samples per day during inspection at the poultry slaughterhouse in the central market of Garoua (Thrusfield, 1994). Samples were taken every day from Monday to Friday from 8:00 am to 10:00 am and consisted of introducing a swab into the animal's cloaca, taking care to ensure that it came into contact with the mucous membrane. The swabs, visibly covered with faecal matter, were transported the samples were kept in an isothermal chamber at 4°C and brought back to the National Veterinary Laboratory (LANAVET) in Garoua within a period not exceeding 4 hours for analysis.

Bacteriological analyses

They were all carried out in the LANAVET diagnostic room according to the NF/EN ISO 6579 (2002) Reference Method for the detection of *Salmonella*. Serotyping was carried out using the slide agglutination technique according to the Kauffmann-White scheme (Guibourdenche *et al.*, 2010) and antibiotic sensitivity was tested using the standard Mueller-Hinton agar diffusion method.

Culture and isolation of *Salmonella* sp.

Each sample was pre-enriched 1:10 with buffered peptone water and incubated 18-20 h at 37°C. 0.1 ml and 1 ml of the pre-enrichment product were then enriched in 10 ml of Rappaport Vassiliadis soybean medium (RVS) and 10 ml of MüllerKauffmann tetrathionate medium (MKTTn), respectively. The RVS medium was incubated at 42°C and the MKTTn at 37°C for 18-24 hours. Approximately 0.5µl of the enrichment was then inoculated onto Hektoen agar

medium, a selective medium used to isolate and differentiate *Salmonella* from other Enterobacteriaceae. After inoculation, the labeled plates were incubated at 37°C for 24 hours.

After incubation, the characteristic *Salmonella* colonies (blue-green to green, with black centers) were identified. The identification was based on the non-use of the carbohydrates present in the medium (salicin, sucrose and lactose) and the production of H_2S , resulting in colonies with a black center, a coloration due to the production of iron sulphide in the presence of ferric citrate. In order to obtain pure colonies, the characteristic *Salmonella* colonies (at least 5) were reseeded on Hektoen medium and incubated at 37°C for 24 hours.

Biochemical identification of *Salmonella* isolates

The characteristic colonies of isolated *Salmonella* were inoculated on sloped Kligler agar and incubated for 24 hours at 37°C to differentiate *Salmonella* from other Gram-negative bacteria. The inoculation was carried out close to the flame, taking care to prick the pellet with the handle carrying the colony and to remove it from the tube by making streaks along the slope. After incubation, tubes with a red slope (lactose-negative), a yellow pellet (glucose-positive) and a black coloration between the pellet and the slope (H_2S production) were used to inoculate the API 10S Galleries.

The API 10S gallery is a series of 10 biochemical tests in the form of microtubes containing substances in dehydrated form. The bacterial suspension with 0.5 mc farland turbidity was used to inoculate the tubes and reconstitute the media without formation of bubbles. For the substrate with CIT character, tube and well are filled with the bacterial suspension. For the other characters, only the tubes were filled with the bacterial suspension and the kerosene oil cups for the characters LDC, ODC, URE and H_2S were filled with the bacterial suspension to create anaerobiosis and keep the volatile ions produced by the reaction in solution. The reconstituted tubes, the gallery placed on its support filled with water, lid on top, was incubated for 24 hours at 37°C. The reactions produced during the incubation period are translated by spontaneous color shifts or revealed by the addition of reagents (James reagent, TDA reagent and TIN1 and TIN2 reagents).

The reading of these reactions was done using the reading table and the identification by referring to the database managed by Biomérieux. For this purpose, the tests were grouped by three successively from left to right, the last triplets being able to include bacterial characters such as morphology, Gram, mobility, oxidase, catalase, which are not studied in the gallery but which are essential for its interpretation. Negative tests are always coded 0 while the code assigned to positive tests varies according to the position of the test in the triplet: 1 for the first test, 2 for the second, 4 for the third. The three triplet results added together, the sums of each triplet read from left to right formed a code of at least seven (07) digits that corresponds to the biochemical profile of the microorganism studied. The comparison of this code with those referenced in the database managed by Biomérieux allowed the identification of the microorganism.

Confirmed *Salmonella* strains were stored at +4°C for further analysis.

Serotyping of *Salmonella* isolates

Based on antigen-antibody reactions, serotyping was carried out by slide agglutination using 24-hour kligler-slope agar culture.

It consisted in checking beforehand that the strains to be studied were not self-agglutinating. To do this, a drop of physiological water was deposited on a clean slide. Subsequently, some of the strain of *Salmonella* sp. was suspended in the drop and spread. By a rocking movement of the slide the agglutination was sought. For the non-self-agglutinating strains, agglutination was then sought successively with the sera anti-O polyvalent (OMA, OMB, OMC), anti-O monovalent then anti-H (HMA, HMB, HMC and H1) following the Kaufmann-white scheme. If the strain possesses the antigen corresponding to the antiserum tested, agglutinates are formed which are visible to the naked eye. Finally, the Kaufmann-White table was used to determine the antigenic formula and to read the serotyping results.

Research on the susceptibility of *Salmonella* strains

The antibiotic susceptibility test was performed according to the Mueller-Hinton agar diffusion method, inoculated with the strain to be tested, for 16 antibiotic discs (BIORAD), as proposed in the recommendations of the Comité de

l'Antibiogramme de la Société Française de Microbiologie (CA-SFM/EUCAST, 2019).

The inoculation was carried out by flooding the entire surface of the medium with a bacterial suspension at 0.5 mc farland turbidity and the cans kept 15 min at room temperature for drying.

The discs were then placed equidistantly from each other, pressing lightly with forceps to ensure contact with the agar.

In order to obtain a pre-diffusion of the antibiotics, the plates were kept at laboratory temperature for 15 min before being incubated in the oven for 24 hours at 37°C.

Antibiotics (Code, disc load) tested are: Amoxicillin + clavulanic acid (AUG, 20/10µg), Amoxicillin (AMX, 20/10µg), Amikacin (AKN, 30µg), Chloramphenicol (C, 30µg), Cefazidime (CAZ, 30µg), Cefaclor (CF, 30µg), Ciprofloxacin (CIP, 5µg), Cephalothin (CL, 30µg), Ceftriaxone (CRO, 30µg), Cefotaxime (CTX, 30µg), Fosfomycin (FM, 200µg), Kanamycin (KMN, 30µg), Nalidixic Acid (NA, 30µg), Netilmicin (NET, 30µg), Tetracycline (TE, 30µg), Ticarcillin (TIC, 75µg).

After incubation, inhibition zones (circles devoid of culture) centered around the antibiotic discs appeared. For each of the antibiotics tested, the inhibition diameter was measured using a caliper and interpreted according to the criteria proposed by the CASFM (CASFM/EUCAST, 2019).

Confirmation by PCR *invA* gene detection of isolated *Salmonella* sp. strains

A standard PCR (standard polymerase chain reaction) was performed to diagnose the strains of *Salmonella* sp.

The invasion gene A (*invA*, 284bp), coding for the pathogenicity of *Salmonella* strains was used as a primer, since it is present in all *Salmonella enterica* strains. Specifically using the oligonucleotide primer sequences 139 (*invA1*) and 141 (*invA2*) (Tab 1).

Table 1 Sequences of primers used.

| Primers | Nucleotide sequences (5' → 3') | References |
|-------------------------|---------------------------------|---|
| 139 (<i>invA1</i>) | GTGAAATTATCGCCACGTTCCGG GCAA | (Rahn et al., 1992; Malorny et al., 2003). |
| 141 (<i>invA2</i>) | TCATCGCACCGTCAAAGGAACC | (Rahn et al., 1992; Malorny et al., 2003). |

DNA extraction

The cultures on sloping kligler agar medium were scraped near the flame using the platinum loop and mixed in cryotubes with 1ml of distilled water. These cultures were then centrifuged for 5min at 13,000g at 4°C. After centrifugation, the supernatant was carefully discarded and the cell deposit, previously washed with a solution of PBS 1X was suspended in 300µl lysis buffer in order to lysis the cell and allow the constituents to emerge. The mixture, incubated for 60 min at 60°C in a water bath was immediately cooled on ice. Following a second centrifugation 5min at 13,000xg at 4°C, the supernatant was carefully transferred to new cryotubes and preserved at -20°C until use. 5µl of this solution was used as DNA.

Preparation of the reaction mixture

The volume and final concentration of each reagent to be used was determined based on the initial concentration of the solution used. All products required for the PCR were thawed on ice. The reaction mixture, commonly known as the mix, was calculated for all the isolates to be tested and prepared by adding the reagents one after the other: ultrapure water, dNTPs, the pair of primers, the PCR buffer and the enzyme (*Taq polymerase*) which is introduced last in the mix. The reaction mixture was then redistributed between the negative control, the positive control and the samples. The DNA to be amplified was subsequently added to the reaction mixture. The PCR reaction was then performed in 0.1ml microtubes, for a reaction volume of 25µl, the composition of which is given in Tab 2.

Amplification conditions

The various DNA amplification steps were performed in successive cycles in a manually programmed thermal cycler. The variable parameters are time, temperature of each amplification step and the number of cycles.

The amplification followed the following program: an initial denaturation at 95°C for 1min, a denaturation at 95°C for 30s, a hybridization at 64°C for 30s, an elongation at 72°C for 30s and a final elongation at 72°C for 4min for the next 38 cycles...

Table 2 Composition of the Mix.

| Constituents | Volume per reaction |
|---|---------------------|
| H ₂ O | 9.6 Ml |
| 10X PCR Buffer | 2.5 Ml |
| dNTP (2mM) | 2.5 µL (200 µM) |
| Primer 139 (<i>invA1</i>) (10pmol/µL) | 1.0 µL (0.4 µM) |
| Primer 141 (<i>invA2</i>) (10pmol/µL) | 1.0 µL (0.4 µM) |
| Magnesium chloride (50mM) | 0.75 µL (1.5 mM) |
| Tag polymerase (5U/µL) | 0.15 µL (0.75 U) |
| BSA (10mg/mL) | 2.5 µL (1 µg/µL) |
| DNA Extract | 5 µL |

DNA electrophoresis on agarose gel

The migration gel is a 3% agarose gel (mass/volume), i.e. 2.5g of agarose powder dissolved in 70ml of TAE 50X solution. The mixture was heated in a microwave oven for about 3 minutes until boiling to obtain a clear homogeneous phase. Then 15µl ethidium bromide was added to the still liquid cooled agarose. The solution obtained was poured into a support on which was fixed a comb forming the wells necessary for the deposition of the amplicons obtained after the PCR. After about 20mn of polymerization, the gel was placed in the electrophoresis cell containing the TAE. With the comb removed, each well was loaded with 12µl of amplicons, previously mixed with 2.5µl of loading buffer (Bromophenol blue 3 mmol.L⁻¹ 0.2 g, Sucrose 1.5 mol.L⁻¹ 5.1 g, Tris. HCl 10 mmol.L⁻¹ 0.01 g [pH 8]). For each comb, one well was also loaded with 12µl of 100 bp DNA molecular weight marker, mixed with loading buffer containing several DNA fragments of known size and serving as a marker ladder. An electrical current of 120 volts was applied to the cell for 45 minutes, with the wells positioned on the cathode side. After the migration, the amplification products were visualized in the darkroom with the help of glasses adapted to the circumstance, the gel deposited on the reading plate generating an ultra violet light. The device that was switched on emitted ultraviolet light, making it possible to distinguish the bands present on the gel. The appearance of fluorescent bands indicates the presence of DNA.

Definition of witnesses

For all amplification reactions, the negative controls consisted of the reaction mixture, ultrapure water (h) and tap water (i). The positive controls were *Salmonella* strains isolated from pigs (f) and humans (g).

Statistical analyses

The sample size was determined according to the formula described by Thrusfield (Thrusfield, 1994). The database was created and managed using Access (Microsoft office corporation 2010). Quantitative data were entered using the Excel software (Microsoft office corporation 2010). The results of the survey as well as those of the antibiotic susceptibility test were synthesized using Microsoft Excel and subjected to a descriptive statistical analysis. This descriptive analysis was based on the use of Person's Chi-square to compare the prevalence of *Salmonella* infections and the rates of germ resistance (Huneau et al., 2007). The statistical significance level was set at 5%.

Table 4 Antigenic characteristics of *Salmonella* sp. strains isolated.

| Origin of the chickens | Serotypes | Antigenic formula | | | Number of isolates | Frequency |
|------------------------|-------------|-------------------|-------|---------|--------------------|-----------|
| | | Ag O | Ag H1 | AgH2 | | |
| Ngong and Gashiga | S. Hadar | 6,8 | z10 | e,n,x | 1 | 20% |
| Adoumri and Baïla | S. Idikan | 1,13,23 | i | 1,5 | 1 | 20% |
| Bé and Bamé | S. Mbandaka | 6,7, 14 | z10 | e,n,z15 | 1 | 20% |
| Bé and Bamé | S. Infantis | 6,7, 14 | r | 1,5 | 1 | 20% |
| Bé and Bamé | S. Anatum | 3,10 | e,h | 1,6 | 1 | 20% |

RESULTS

Characterization of samples

The study involved 400 samples of cloacal swab products, collected at the poultry slaughterhouse in Garoua's central market. This facility has a daily slaughter capacity of more than 50 chickens, depending on inputs from markets held in neighboring villages.

Colonies obtained in culture

More or less isolated colonies are present on Hektoen agar (Figure 1A), which has taken on a salmon (pinkish-orange) color, indicating that the medium has become acidified as a result of the fermentation of one of the carbohydrates (lactose, sucrose and salicin) present in the medium by microorganisms other than *Salmonella* and *Shigella*. Isolated colonies with green coloration with black centers, a typical *Salmonella* phenotype due to the production of hydrogen sulfide in the presence of ferric citrate contained in the medium, gave after transplantation pure *Salmonella* colonies (Figure 1B).

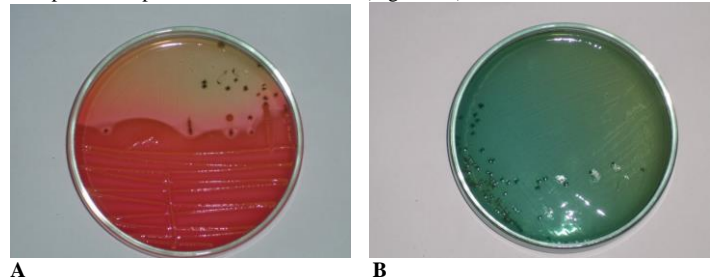


Figure 1 Figure 1: Growth on Hektoen agar medium A) Colonies on Hektoen medium B) Pure colony of *Salmonella* sp. on Hektoen medium.

Prevalence of *Salmonella* infections in chickens at the slaughterhouse

Out of the 400 samples collected, five (05) *Salmonella* strains were isolated, representing a prevalence of *Salmonella* infections of 1.3% observed at the poultry slaughterhouse in the central market of Garoua. One strain was isolated from chickens from Ngong and Gashiga (1.25%), another from chickens from Adoumri and Baïla (1.25%), and three (03) from chickens from Bé and Bamé (3.75%) (Tab 3).

Table 3 Prevalence of *Salmonella* infections.

| Origin of the poultry | Samples | | |
|-----------------------|------------|-----------|----------------|
| | Analysed | Positives | Prevalence (%) |
| Ngong and Gashiga | 80 | 1 | 1.25 |
| Babla and Ouro Labo | 80 | 00 | 00 |
| Bé and Bamé | 80 | 03 | 3.75 |
| Adoumri and Baïla | 80 | 01 | 1.25 |
| Guide | 80 | 00 | 00 |
| Total | 400 | 05 | 1.3 |

Serotypes of *Salmonella* strains

Serotyping of *Salmonella* sp. strains resulted in the identification of five (05) different serotypes (Tab 4), including: S. Hadar, S. Idikan, S. Mbandaka, S. Infantis and S. Infantis. Anatum (n=1 and 20% each).

Antibiotic susceptibility of *Salmonella* strains

With regard to the critical concentrations and diameters observed in Figure 2, are considered as:

- Sensitive (S) means strains for which the MIC of the antibiotic tested is less than or equal to the low critical concentration, which is equivalent to a diameter greater than or equal to the critical diameter.
- Resistant (R), strains against which the MIC of the tested antibiotic is above the high critical concentration, corresponding to a diameter strictly below the critical diameter.
- Intermediate Sensitivity (I), the strains for which the MIC of the antibiotic tested and the corresponding diameter is between the two critical concentrations and the two critical diameters.



Figure 2 Antibiogram of *Salmonella* sp.

Resistance prevalences of *Salmonella* sp.

Of the 5 *Salmonella* strains isolated, all strains (100%) were tested resistant to 4 of the 16 antibiotics used, representing a prevalence of resistance of 25%. The prevalences of resistance of *Salmonella* strains to the antibiotics used are recorded in Tab 5.

From Tab 5, we can observe a very good sensitivity of *Salmonella* strains (100%) towards 12 antibiotics out of the 16 tested: Amoxicillin + clavulanic acid, Amoxicillin, Chloramphenicol, Ceftazidime, Cefaclor, Ciprofloxacin, Ceftriaxone, Cefotaxime, Fosfomycin, Nalidixic acid, Netilmicin, Ticarcillin. Low to medium sensitivity was observed with 3 antibiotics including Tetracycline (20%), Amikacin (40%), Kanamycin (60%). The highest prevalence of resistance was observed with Cephalothin (100%).

Table 5 Sensitivity of *Salmonella* strains.

| Origin of the chickens Staff | Ngong and Gashiga | | Bé and Bamé | | Adoumri and Baïla | |
|---------------------------------|-------------------|----------|-------------|------------|-------------------|----------|
| | 01 | | 03 | | 01 | |
| Categories | S (%) | R+I (%) | S (%) | R+I (%) | S (%) | R+I (%) |
| Amoxicillin + clavulanic acid | 01 (100) | 00 (00) | 03 (100) | 00 (00) | 01 (100) | 00 (00) |
| Amoxicillin | 01 (100) | 00 (00) | 03 (100) | 00 (00) | 01 (100) | 00 (00) |
| Amikacine | 01 (100) | 00 (00) | 01 (33.33) | 02 (66.67) | 01 (100) | 00 (00) |
| Chloramphenicol | 01 (100) | 00 (00) | 03 (100) | 00 (00) | 01 (100) | 00 (00) |
| Ceftazidine | 01 (100) | 00 (00) | 03 (100) | 00 (00) | 01 (100) | 00 (00) |
| Céfaclor | 01 (100) | 00 (00) | 03 (100) | 00 (00) | 01 (100) | 00 (00) |
| Ciprofloxacin | 01 (100) | 00 (00) | 03 (100) | 00 (00) | 01 (100) | 00 (00) |
| Cephalothin | 00 (00) | 01 (100) | 00 (00) | 03 (100) | 00 (00) | 01 (100) |
| Ceftriaxone | 01 (100) | 00 (00) | 03 (100) | 00 (00) | 01 (100) | 00 (00) |
| Céfotaxime | 01 (100) | 00 (00) | 03 (100) | 00 (00) | 01 (100) | 00 (00) |
| Fosfomycin | 01 (100) | 00 (00) | 03 (100) | 00 (00) | 01 (100) | 00 (00) |
| Kanamycin | 00 (00) | 01 (100) | 01 (33.33) | 02 (66.67) | 01 (100) | 00 (00) |
| Nalidixic acid | 01 (100) | 00 (00) | 02 (66.67) | 01 (33.33) | 01 (100) | 00 (00) |
| Netilmicine | 01 (100) | 00 (00) | 03 (100) | 00 (00) | 01 (100) | 00 (00) |
| Tetracycline | 01 (100) | 00 (00) | 03 (100) | 00 (00) | 00 (00) | 01 (100) |
| Ticarcillin | 01 (100) | 00 (00) | 03 (100) | 00 (00) | 01 (100) | 00 (00) |

Resistance Phenotypes of *Salmonella* sp.

Among the 5 *Salmonella* serotypes isolated, all showed resistance to at least one antibiotic. Tab 6 presents the resistance phenotypes of the different serotypes identified.

Of the isolated serovars, *S. Mbandaka* was resistant only to cefalotin. Strains of *S. Hadar* and *S. Idikan* were resistant to Kanamycin and Tetracycline, respectively, in addition to Cefalotin. In addition, the serotypes *Infantis* and *Anatum* each showed resistance to 3 antibiotics (Kanamycin, Amikacin and Cefalotin) (Tab 6).

Table 6 Resistance Phenotypes of *Salmonella* Serotypes Isolated

| Serotypes | Tested | Number of strains (%) | | | Phenotypes |
|--------------------|--------|-----------------------|----------------|-----------------|---------------|
| | | Sensitive | Mono resistant | Multi resistant | |
| <i>S. Hadar</i> | 1 | 0 (00) | 0 (00) | 1 (100) | CEF, KMN |
| <i>S. Idikan</i> | 1 | 0 (00) | 0 (00) | 1 (100) | CEF, TE |
| <i>S. Mbandaka</i> | 1 | 0 (00) | 1 (100) | 0 (00) | CEF |
| <i>S. Infantis</i> | 1 | 0 (00) | 0 (00) | 1 (100) | AKN, CEF, KMN |
| <i>S. Anatum</i> | 1 | 0 (00) | 0 (00) | 1 (100) | AKN, CEF, KMN |

Legend: *S.* *Salmonella*; KMN: Kanamycin; AKN: Amikacin; CEF: Cefalotin; TE: Tetracycline

Genotypic profile of identified *Salmonella* strains

The *invA* invasion gene was amplified by PCR from *Salmonella* sp. DNA in the presence of primers 139 (*invA1*) and 141 (*invA2*). Figure 3 shows the migration of the amplified bands ranging from 250 to 300 bp of the molecular weight marker to approximately 284 bp.

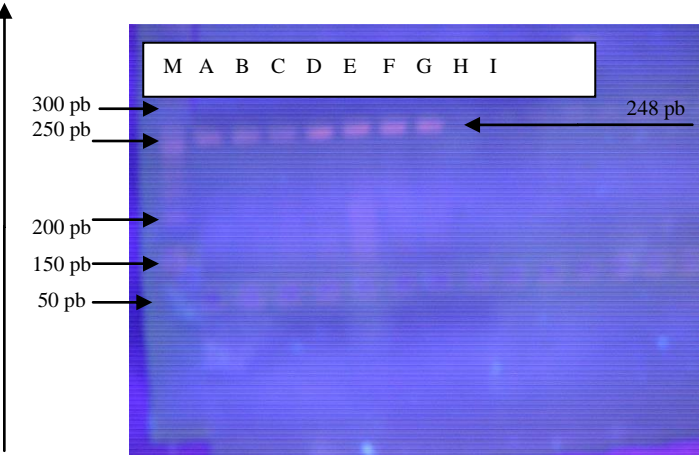


Figure 3 Figure 3: 3% agarose gel electrophoresis of PCR products. **M:** 50 bp molecular weight marker; **A:** *S. Hadar*; **B:** *S. Infantis*; **C:** *S. Idikan*; **D:** *S. Mbandaka*; **E:** *S. Anatum*; **F:** positive control (*Salmonella* strain isolated from pigs); **G:** positive control (*Salmonella* strain isolated from humans); **H:** negative control (ultrapure water); **I:** negative control (tap water).

DISCUSSION

The study estimated the prevalence of *Salmonella* infections in traditional chickens slaughtered at Garoua's central market for this study period at 1.3%. This level of contamination is in line with the prevalence observed within the European Community in broilers, between 0% and 18% (AFSSA, 2006). These figures are obtained from different survey and surveillance programs and reflect the differences in sampling and sampling schemes used from one country to another (AFSSA, 2006, Direction des services vétérinaires, 2003).

In addition, prevalence was estimated in France at 69.8% (Rose et al., 1999), in Turkey at 41.3% (Carli et al., 2001) and in Senegal in 2000-2001 at 28.6% (Cardinale et al., 2004). In recent years, and probably thanks to the performance of European Community control programs, *Salmonella* contaminations seem to be declining in most European states.

Non-typical *Salmonella* strains are widely distributed in nature, colonizing a wide range of animals, including mammals, amphibians, reptiles, birds and insects (Butaye, 2006). However, there is little information on environmental reservoirs and modes of contamination of non-typical *Salmonella* strains, particularly in the African context (Morpeh et al., 2009).

From Tab 5, it appears that sample size is significantly associated with the isolation of *Salmonella* sp. This has been confirmed by the studies of Mollenhorst et al. (2005) in the Netherlands and Namata et al. (2006) in Belgium.

The serovars identified in the present study are very similar to those in a European community study, which reports that the most frequent serovars in broilers are *S. Enteritidis*, *S. Typhimurium*, *S. Infantis*, *S. Mbandaka* and *S. Hadar*, (Devos, 2007). *S. Hadar* is also the most frequently isolated from the poultry industry in Canada (Chambers et al., 1998) and France (Rose et al., 1999; Brisabois et al., 2006). In a report by the European Food Safety Authority (EFSA), *S. Hadar* was reported to be the most common serotype in the broiler industry, followed by *S. Infantis* and *S. Infantis*. Virchow (EFSA, 2007).

No strain of *Salmonella* Typhi, responsible for typhoid fever in humans, has been isolated. This would be due to the fact that *Salmonella* Typhi is considered as a parasite of the human intestine and its presence elsewhere would only be due to faecal contamination (Bornet, 2000).

The interpretative reading of the antibiogram, based on the knowledge of resistance phenotypes, leads in some cases to transform a result initially categorized as S into an I or R result because of a risk of therapeutic failure (CA-SFM, 2010). Moreover, for some bacterial-antibiotic pairs, despite a "sensitive"

categorization, the increased risk of in vivo selection of resistant mutants justifies a special commentary for the clinician (CA-SFM/EUCAST, 2016).

Therefore, the intermediate strains tested (I) were categorized as resistant (R) in the present study. Thus, out of the 05 strains isolated, all were tested resistant to at least 1 antibiotic, i.e. an overall prevalence of resistance of 25%. This fairly high prevalence of resistance in bacteria of avian origin is in line with those reported in other studies in Algeria (Elgroud et al., 2009), France (Sanders et al., 2002) and Ethiopia (Tibaijuka et al., 2007), but remains above the results of a previous study conducted in Cameroon (Ateudjieu et al., 2018); which attests to the evolution of antimicrobial resistance over time in Cameroon.

Tab 4 shows the resistance of *Salm*), kanamycin and cephalothin. This would be due to the availability on the market of these low-cost antibiotics and their sometimes anarchic use, without precise *onella* strains to tetracycline (oxytetracycline group), amikacin (aminoside group diagnosis and in insufficient doses or in overdosage in the veterinary field both in food and for the treatment of bacterial infections (Ungemach et al., 2006). At any given time, this overuse of antibiotics imposes selection pressure on bacterial populations; this is the case with tetracyclines, which are the most widely used food additives in some parts of the world (Helmuth, 2000; Ungemach et al., 2006).

Indeed, the exclusive and intensive use of an antibiotic could select resistant strains (Chauvin, 2009). It should also be noted that, in the natural environment, bacteria can harbour resistance genes derived from the use of antibiotics in animals (Health Canada, 2005). It is important to note that enterobacteria have an obvious capacity to acquire and exchange genes carrying resistance factors and the intestinal flora provides an extraordinary opportunity for the circulation of genetic information between bacteria (Martel et al., 2002).

This resistance can be attributed to the acquisition of a foreign gene through horizontal transfer of plasmids, transposons or integrons (Gebreyer and Thakur, 2005), between donor and recipient bacteria by transformation, transduction and conjugation (Schwarz and Chaslus-Dancla, 2001), or to a mutation following an alteration of the bacterial genome, but the latter remains rare and spontaneous (Catry et al., 2003).

Salmonella antibiotic resistance reduces the therapeutic and prophylactic efficacy of antibiotics used in veterinary medicine and poses a problem in the case of zoonoses, since these resistant bacteria can be transmitted to humans (Helmuth, 2000).

However, all strains remained sensitive to Ciprofloxacin, Amoxicillin, Chloramphenicol, Ceftriaxone and Nalidixic Acid.

In addition, studies by Wang et al. (2010) and Jiang et al. (2011) in China on avian clinical cases showed prevalences of resistance to chloramphenicol of 74% and 50.2%, respectively. In veterinary medicine, its broad spectrum of activity, particular efficacy and moderate cost made chloramphenicol a widely used antibiotic, but the risk of induction of bone marrow aplasia that it presents was the reason for its ban in production animals (Puyt, 2004). Thus, the recorded sensitivity could be an indicator of its non-use by poultry farmers in Cameroon.

It follows from the results of this study that *Salmonella* strains are still largely sensitive to quinolones (Martel and Chaslus-Dancla, 2000), with 0% resistance for nalidixic acid and for ciprofloxacin, which is a recent molecule.

The multiresistance of the serovars Infantis (100%) and Anatum (100%) involved 3 antibiotics, i.e. the AKN-CEF-KMN phenotype and that of Hadar (100%) and Idikan (100%) 2 antibiotics each, i.e. the CEF-KMN and AKN, CEF-TE phenotypes. Similarly, multi-resistant *Salmonella* strains of avian origin were isolated by Tibaijuka et al. (2007). These data corroborate our results and seem to indicate a recrudescence of multiresistance in *Salmonella* serovars frequently isolated from poultry.

The 284 bp bands observed in Figure 3 correspond to the sequence encoding the *invA* gene amplified in the presence of specific primers (139 (*invA1*) and 141 (*invA2*)) from *Salmonella* sp. DNA. (Rahn et al., 1992). Indeed, the *invA* gene sequence is located on *Salmonella* sp. pathogenicity islet 1 and codes for the protein of the type III secretion system (Collazo and Galan, 1997). These genomic islets group together numerous genes involved in the same function such as metabolism, adaptation to a specific environment, antibiotic resistance or virulence (Hacker and Kaper, 2000). In addition, the *Salmonella* PCR technique has a sensitivity of 99.6% and a specificity of 100% for the *invA* gene (Malorny et al., 2003), making this method highly reliable.

CONCLUSION

During the targeted period and at the poultry slaughterhouse of the central market of Garoua in the Northern Region of Cameroon, five (5) strains of *Salmonella*

were isolated. This made it possible to identify for the first time the trends in *Salmonella* contaminations in the traditional chicken industry in the region. It clearly appears that the bacterial resistance observed in this study reflects the previous intensive use of antibiotics that caused the acquisition of foreign genes through the horizontal transfer of plasmids, transposons or integrons between donor and recipient bacteria, by transformation, transduction and conjugation, or mutation following an alteration of the bacterial genome. Overall, the strains were often resistant to at least one antibiotic, but mainly to older molecules such as Tetracyclines, or fairly common ones such as Kanamycin. Several phenotypes of antibiotic resistance were identified, suggesting epidemiological links at different levels of the poultry industry in different villages. It would therefore not be absurd to say that the phenomenon of antibiotic resistance would regress with the cessation of the use of additives in veterinary medicine. The use of additives in food, such as tetracyclines, is at the origin of antibiotic resistance in veterinary medicine, which can be transmitted to humans. In addition, since the majority of antibiotic-resistant strains of *Salmonella* are still associated with more serious infections (septicemia), higher hospitalization rates in human medicine and a significant drop in animal productivity, it would be crucial and urgent to increase the monitoring of antibiotic use.

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REFERENCES

- Agence Française de Sécurité Sanitaire des Aliments (AFSSA) (2006). Veterinary uses of antibiotics, bacterial resistance and consequences for human health. Afssa, Printing: Bialec, Nancy (France). 214 p.
- Ateudjieu, J., Bit'a'a, L.B., Guenou, E., Chebe, A.N., Chukuwchindun, B.A., Goura, A.P. & Bissec, A.C.Z.K. (2018). Profile and antibiotic susceptibility of pathogenic bacteria associated with diarrhea in patients consulting at the Regional Annex Hospital of Kousseri, Far North Cameroon. *Pan African Med. J.*, 29: 170. <https://doi.org/10.11604/pamj.2018.29.170.14296>
- Bornet, G. (2000). *Salmonella*-free chicken: Myth or reality? Review of Veterinary Medicine. 151, 12: 1083-1094.
- Brisabois, A., Danan, C., Frémy, S., Granier, S., Moury, F., Oudart, C., Piquet, C. & Pires Gomes, C. (2006). Inventory of the *Salmonella* network; Serotyping and antibiotic susceptibility, 2004 data. Editions AFSSA, Maisons Alfort, France. 114 p.
- Butaye, P., Michael, G. B., Schwarz, S., Barrett, T. J., Brisabois, A., & White, D. G. (2006). The clonal spread of multidrug-resistant non-typhi *Salmonella* serotypes. *Microbes and Infection*, 8(7), 1891-1897. <https://doi.org/10.1016/j.micinf.2005.12.020>
- Cardinale, E., Tall, F., Guèye, E., Cisse, M., & Salvat, G. (2004). Risk factors for *Salmonella enterica* subsp. *enterica* infection in senegalese broiler-chicken flocks. *Preventive Veterinary Medicine*, 63(3-4), 151-161. <https://doi.org/10.1016/j.prevetmed.2004.03.002>
- CARLI, K. T., EYIGOR, A., & CANER, V. (2001). Prevalence of *Salmonella* Serovars in Chickens in Turkey. *Journal of Food Protection*, 64(11), 1832-1835. <https://doi.org/10.4315/0362-028x-64.11.1832>
- Catry, B., Laevens, H., Devriese, L. A., Opsomer, G., & de Kruif, A. (2003). Antimicrobial resistance in livestock. *Journal of Veterinary Pharmacology and Therapeutics*, 26(2), 81-93. <https://doi.org/10.1046/j.1365-2885.2003.00463.x>
- Chambers, Bisailon, Labbe, Y., Poppe, C., & Langford, C. F. (1998). *Salmonella* prevalence in crops of Ontario and Quebec broiler chickens at slaughter. *Poultry Science*, 77(10), 1497-1501. <https://doi.org/10.1093/ps/77.10.1497>
- Chauvin, C. (2009). Usage des antibiotiques et résistance bactérienne en élevage de volaille (PhD thesis), Université Rennes 1, 25 p.
- Collazo, C. M., & Galán, J. E. (1997). The invasion-associated type-III protein secretion system in *Salmonella* – a review. *Gene*, 192(1), 51-59. [https://doi.org/10.1016/S0378-1119\(96\)00825-6](https://doi.org/10.1016/S0378-1119(96)00825-6)
- Comité de l'antibiogramme de la Société française de microbiologie (CA-SFM), 2010. Recommendations. January 2010 edition, 50 p.
- Comité de l'antibiogramme de la Société française de microbiologie, European Committee on Antimicrobial Susceptibility Testing (CA-SFM/EUCAST) (2016). Recommendations 2016. Version 1.0 January 2015, 117 p.
- Comité de l'antibiogramme de la Société française de microbiologie, European Committee on Antimicrobial Susceptibility Testing (CA-SFM/EUCAST) (2019). Recommendations 2019. V.1.0 January, 144 p.
- Devos, N. (2007). *Salmonella*. Survey in European farms. La Semaine Vétérinaire, n° 1267. 24 p.
- Directorate of Veterinary Services, Ministry of Agriculture and Rural Development (2003). Bulletin Sanitaire Vétérinaire Year 2003. Algeria. 6 p.
- Elgroud, R., Zerdoumi, F., Benazzouz, M., Bouzitouna-Bentchouala, C., Granier, S. A., Frémy, S., ... Millemann, Y. (2009). Characteristics of *Salmonella* Contamination of Broilers and Slaughterhouses in the Region of Constantine (Algeria). *Zoonoses and Public Health*, 56(2), 84-93. <https://doi.org/10.1111/j.1863-2378.2008.01164.x>
- European Food Safety Authority (2007). Report of the Task Force on Zoonoses Data Collection on the Analysis of the baseline survey on the prevalence of *Salmonella* in broiler flocks of *Gallus gallus*, in the EU, 2005-2006 - Part A: *Salmonella* prevalence estimates. EFSA Journal, 5(3), 98r. <https://doi.org/10.2903/j.efsa.2007.98r>
- Gebreyes, W. A., & Thakur, S. (2005). Multidrug-Resistant *Salmonella enterica* Serovar Muenchen from Pigs and Humans and Potential Interserovar Transfer of Antimicrobial Resistance. *Antimicrobial Agents and Chemotherapy*, 49(2), 503-511. <https://doi.org/10.1128/aac.49.2.503-511.2005>
- Guibourdenche, M., Roggentin, P., Mikoleit, M., Fields, P. I., Bockemühl, J., Grimont, P. A. D., & Weill, F.-X. (2010). Supplement 2003-2007 (No. 47) to the White-Kauffmann-Le Minor scheme. *Research in Microbiology*, 161(1), 26-29. <https://doi.org/10.1016/j.resmic.2009.10.002>
- Hacker, J., & Kaper, J. B. (2000). Pathogenicity Islands and the Evolution of Microbes. *Annual Review of Microbiology*, 54(1), 641-679. <https://doi.org/10.1146/annurev.micro.54.1.641>
- Health Canada (2005). Prevention of Salmonellosis. Retrieved October 23, 2009.
- Helmuth R., 2000. Antibiotic Resistance in *Salmonella*. In: *Salmonella* in Domestic Animals, edited by Wray C. And Wray A. CABI publishing. London, UK. pp. 89-106.
- Huneau, S. A., Chemaly, M., Petetin, I., Rouxel, S., Lalande, F. & Le Bouquin, S. (2007). Multidimensional descriptive analysis of layer farms contaminated by *Salmonella* spp: Search for risk factor hypotheses. Septièmes Journées de la Recherche Avicole, Tours, 28-29, March 2007, pp. 505-509.
- Malorny, B., Hoorfar, J., Bunge, C., & Helmuth, R. (2003). Multicenter Validation of the Analytical Accuracy of *Salmonella* PCR: towards an International Standard. *Applied and Environmental Microbiology*, 69(1), 290-296. <https://doi.org/10.1128/aem.69.1.290-296.2003>
- Martel, J. L., & Chaslus-Dancla, E. (2000). Practical aspects of antibiotic resistance in veterinary medicine. *Medicine and Infectious Diseases*, 30, s173 - s177. [https://doi.org/10.1016/S0399-077X\(00\)89086-1](https://doi.org/10.1016/S0399-077X(00)89086-1)
- Martel, J.-L., Jouy, É., Meunier, D., Kobisch, M., Coudert, M., & Sanders, P. (2002). Methodology of the national network for the surveillance of antibiotic resistance in the main pathogenic bacteria of production animals (RESAPATH). *Bulletin of the French Veterinary Academy*, (3-4), 259. <https://doi.org/10.4267/2042/61541>
- Morpeth, S. C., Ramadhani, H. O., & Crump, J. A. (2009). Invasive Non-Typhi *Salmonella* Disease in Africa. *Clinical Infectious Diseases*, 49(4), 606-611. <https://doi.org/10.1086/603553>
- Puyt, J. D. (2004). Médicaments anti-infectieux en médecine vétérinaire, document intended for graduate students of the Ecoles Nationales Vétérinaires, 215 p.
- Rahn, K., De Grandis, S. A., Clarke, R. C., McEwen, S. A., Galán, J. E., Ginocchio, C., ... Gyles, C. L. (1992). Amplification of an *invA* gene sequence of *Salmonella typhimurium* by polymerase chain reaction as a specific method of detection of *Salmonella*. *Molecular and Cellular Probes*, 6(4), 271-279. [https://doi.org/10.1016/0890-8508\(92\)90002-f](https://doi.org/10.1016/0890-8508(92)90002-f)
- Rose, N., Beaudeau, F., Drouin, P., Toux, J., Rose, V., & Colin, P. (1999). Risk factors for *Salmonella enterica* subsp. *enterica* contamination in French broiler-chicken flocks at the end of the rearing period. *Preventive Veterinary Medicine*, 39(4), 265-277. [https://doi.org/10.1016/S0167-5877\(99\)00002-1](https://doi.org/10.1016/S0167-5877(99)00002-1)
- Rostagno, M. H., Wesley, I. V., Trampel, D. W., & Hurd, H. S. (2006). *Salmonella* Prevalence in Market-Age Turkeys On-Farm and at Slaughter. *Poultry Science*, 85(10), 1838-1842. <https://doi.org/10.1093/ps/85.10.1838>
- Salm-Surv, G. (2005). A WHO network for the surveillance of foodborne diseases, in INFOSAN Information Note No. 6/- 75: 236-237.
- Sanders, P., Gicquel, M., Humbert, F., Perrin-Guyomard, A., & Salvat, G. (2002). Surveillance plan for antibiotic resistance in indicator bacteria isolated

from the intestinal flora of pigs and poultry 1999-2001. Bulletin of the French Veterinary Academy, (3-4), 267. <https://doi.org/10.4267/2042/61542>

Schwarz, S., & Chaslus-Dancla, E. (2001). Use of antimicrobials in veterinary medicine and mechanisms of resistance. Veterinary Research, 32(3/4), 201–225. <https://doi.org/10.1051/vetres:2001120>

Thrusfield, M. (1994). Biological markers in epidemiology. Preventive Veterinary Medicine, 21(3), 273. [https://doi.org/10.1016/0167-5877\(94\)90025-6](https://doi.org/10.1016/0167-5877(94)90025-6)

Tibaijuka, B., Molla, B., Hildebrandt, G., Kleer, J., & Salah, W. (2007). Antimicrobial resistance to *Salmonellae* isolated from retail raw chicken meat and giblets in Ethiopia. Bulletin of Animal Health and Production in Africa, 50(2). <https://doi.org/10.4314/bahpa.v50i2.32746>

Ungemach, F. R., Müller-Bahr, D., & Abraham, G. (2006). Guidelines for prudent use of antimicrobials and their implications on antibiotic usage in veterinary medicine. International Journal of Medical Microbiology, 296, 33–38. <https://doi.org/10.1016/j.ijmm.2006.01.059>

World Health Organization (WHO) (2000). WHO Surveillance Programme for Control of Foodborne Infections and Intoxications in Europe. Seventh Report on Surveillance of Foodborne Disease in Europe. 32 p.

World Health Organization Food, Agriculture Organization of the United Nations (WHO/FAO) (2009). FAO/WHO expert meeting on the risks of *Salmonella* and *Campylobacter* in chicken meat. FAO Headquarters, Rome, Italy. pp. 1-4.