# Antibiotic Resistance Profile of *Salmonella* Strains Isolated at the National Clinical Biology and Public Health Laboratory in Bangui, Central African Republic

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## Abstract

In Africa, each year, there are estimated to be more than 91 million cases of salmonellosis and 137,000 cases of death. The problem of antibiotic resistance in *Salmonella* strains is a threat to public health. The objective of this study is to evaluate the antibiotic resistance profile of *Salmonella* strains isolated in biological products analyzed at the National Laboratory of Clinical Biology and Public Health (NLCBPH) in Bangui. This is, therefore, a cross-sectional study with a descriptive aim, running from January to December 2022. It focused on the strains of *Salmonella* isolated and identified in stools, urines, and blood samples. For each strain of *Salmonella* isolated, an antibiogram was carried out following the recommendations of the French Society of Microbiology (CASFM, 2022). A total of 93 strains of *Salmonella* have been recorded. The age group 0 - 9 years was 29% and that of >50 years was 11%. The median age of patients was 30 years with a minimum of 1 and a maximum of 78 years. The female gender was more represented at 52.69% than the male gender at 47.31%, *i.e.* a sex ratio of 0.89 (M/F). *Salmonella* strains were much more isolated in stools at 62% followed by urines at 29% and blood at 6%. *Salmonella arizonae* strains were more represented with 52%. *Salmonella* strains have a resistance rate to Tetracycline of 62.37% followed by Penicillins of 50%. The rate of multi-antibiotic resistance of the *Salmonella* strains isolated represented 48.38%. *Salmonella* spp. strains were multi-resistant at 58.69% followed by *Salmonella arizonae* strains at 47.91%. There is a signif-

icant association between the different families of antibiotics and *Salmonella* strains (p < 0.05). According to the results obtained, Penicillins, Phenicoles, and Cyclins had a high rate of resistance on *Salmonella* strains. No strain-producing Broad Spectrum Beta-lactamase has been isolated. *Salmonella* strains represent a zoonotic health danger, constitute a public health problem and remain a current subject. This germ is resistant to the antibiotics used. It is, therefore, essential to emphasize monitoring the resistance of these germs in the Central African Republic (CAR) to improve the health of the population.

## Keywords

*Salmonella*, Resistance, Antibiotics, Central African Republic

## 1. Introduction

Salmonellosis remains today the most common foodborne illness in the world. They are caused by bacteria of the *Salmonella genus*, which are facultative intracellular gram-negative bacilli of the *Enterobacteriaceae* family. There are several species of which only the *enterica*, *arizonae*, and *arizona* subspecies are pathogenic for humans. The *salamae* and *houteanae* subspecies are rather specific to reptiles and are not very widespread [1]. In 1888, Gäertner *et al.* isolated *Salmonella enteritidis* and established a link between human salmonellosis and food consumption [2]. Non-typhoid *Salmonella* causes 3.4 million infections and 681,000 deaths worldwide. According to the WHO, each year, 1 in 10 people become ill from consuming contaminated food and an estimated 550 million people become ill, including 220 million children under the age of 5 [3]. There are an estimated 3 million deaths and numerous economic losses due to *Salmonella* infection [4]. In Africa, the number of deaths is estimated at more than 91 million each year [5]. Most cases of non-typhoid *Salmonella* isolated in Africa are *Salmonella typhimurium* or *Salmonella enteritidis* [6]. In South Africa, other research has reported *Salmonella* using serotypes [7]; in Ethiopia, *Salmonella concord* [8] and in Mali, *Salmonella dublin* [9]. Public Health France estimates that annually 183,002 cases of symptomatic *Salmonella* spp. infections have a food origin [10]. In human pathology, salmonellosis brings together two main types of conditions: gastroenteritis and typhoid and paratyphoid fevers. More than 2500 *Salmonella* serotypes are considered pathogenic for humans [11].

Antibiotics that were used to treat these bacteria are becoming more and more ineffective (antibiotic resistance of bacteria). Thus, antibiotic resistance in *Salmonella* is often observed due to inappropriate use of antibiotics. According to Public Health France, at the molecular level, antibiotic resistance results either from chromosomal mutations (modification of genes already present) or from the acquisition of plasmids, which are transmitted from bacteria to bacteria. Chromosome resistance only affects one antibiotic or family of antibiotics at a time. Plasmid resistance is the most widespread (80% of acquired resistance) and can

concern several antibiotics, or even several families of antibiotics [12].

*Salmonella* is likely to develop acquired resistance by two mechanisms: by the transfer of resistance genes, of plasma origin or linked to transposons, which is the most common, and by mutation, which is rare. However, bacteria in the natural environment may harbor resistance genes derived from the use of these drugs in humans and animals [13]. In Mali, according to a study carried out at the research laboratory in 2017 showed that out of 54 strains of *Salmonella* isolated, 31.91% were resistant to nalidixic acid, 20.98% to Tobramycin, 18.51% to Amoxicillin and 13.20% Co-trimoxazole [14]. In 2020 in Burkina Faso, resistance to cefixime, cefepime was 67%, and to ceftriaxone, cefotaxime was 56%, strains of *Salmonella* spp. [15]. In 2015, WHO recommended the development of national action plans to combat antimicrobial resistance [16]. If no appropriate action is taken to halt its progress, antimicrobial resistance will cost an estimated 10 million lives and an estimated US$100 billion per year by 2050 [17].

In CAR, previous studies carried out by Georges-Courbot *et al.* as well as Mossoro-Kpinde *et al.* on the antibiotic resistance of *Salmonella* strains had shown remarkable ineffectiveness to ceftriaxone in 2008 and 2009 respectively. On the other hand, another study in 2010 and 2011 respectively showed resistance to ceftriaxone of 17% and 43% on *Salmonella* strains [18] [19] [20] [21] [22]. The problem of antibiotic resistance of strains of pathogenic bacteria, including *Salmonella*, is a threat to public health in the CAR, as it compromises the prevention and effective treatment of a growing number of infections. It is in this context that this study was carried out, the objective of which was to evaluate the antibiotic resistance profile of *Salmonella* strains isolated in biological products analyzed at the NLCBPH.

## 2. Methodology

This was a cross-sectional study with a descriptive aim, running from January 1 to December 31, 2022, at the NLCBPH. The sampling was exhaustive and covered all stools samples, urines, and blood in patients from different reference health facilities in Bangui (Community University Hospital Center, Pediatric University Hospital Center), Sino Central African Friendship University Hospital Center) for stools culture analyses, Cytobacteriological Examination of Urine and Haemoculture.

A form was used to collect data on sociodemographic characteristics (age, sex) and the results of the biological analysis.

### 2.1. Biological Diagnosis

The stool samples were sent to the laboratory as soon as they were issued in sterile jars. Preenrichment was carried out in sodium selenite broths and incubated at 37°C for 24 hours. The sodium selenite broths were then subcultured in Hektoen medium and incubated at 37°C for 24 h. The urine collected in sterile pots was inoculated on Cystine Lactose Electrolyte Deficient agar medium, Bromo Cresol Pourpre media, and incubated at 37°C for 24 hours.

The blood collected using a syringe (10 ml) was pre-enriched in brain heart broth medium and incubated at 37˚C for 24 hours, then transplanted into chocolate agar medium and incubated at 37˚C for 24 hours.

The identification of *Salmonella* strains in the different culture media was based on the macroscopic description of the colonies, followed by microscopic examination and the identification of bacterial species using the API 20 E gallery.

Macroscopic aspects allowed us to note the color, size, and shape of the colonies depending on the culture media used. On Hektoen agar medium, the colonies are green with a black center, 2 to 4 mm in diameter, smooth, rounded, and with regular contours. On Cled agar medium, the colonies are green, and blue and do not close lactose. On Chocolate agar medium, the colonies have a colorless or almost dirty-white appearance, shiny, 1 to 3 mm in diameter.

The microscopic aspect made it possible to note after the Gram stain, the morphology of the bacteria.

*Salmonella* strains were identified based on biochemical characteristics. The API 20 gallery made it possible to highlight enzymatic activities and carbohydrate fermentations.

The antibiogram was carried out following the recommendations of the French Society of Microbiology (CASFM, 2022). A total of fifteen antibiotics (Biorad, Marnes-la-coquette, France) were tested: Cefepime (FEP 30 µg), Cefotaxime (CTX: 30 µg), Ceftriaxone (CRO: 30 µg), Cefixime (CFM: 5 µg); Cefoxitin (FOX: 30 µg): Amoxicillin + clavulanic acid (AMC: 20 - 10 µg), Ampicillin (AM: 30 µg), Ciprofloxacin (CIP: 5 µg), Ofloxacin (OFX: 5 µg), Pefloxacin (PEF: 5 µg), Amykacin (AK: 30 µg), Kanamycin (K: 30 IU), Tobramycin (TOB: 10 µg), Chloramphenicol (C: 30 µg), Tetracycline (TE: 30 µg), Imipeneme (IMP: 10 µg).

From 18 - 24 hour cultures of *Salmonella* strains, suspensions equivalent to the McFarland 0.5 standard (approximately $10^8$ CFU/mL) were prepared in sterile physiological water. Hundredfold dilutions of these suspensions were made in distilled water and put in Muller Hinton environment. The antibiotic disks were deposited using a disk dispenser and the plates were incubated at 37˚C for 24 hours. The inhibition diameters were measured using a calibrated measuring instrument and interpreted as sensitive or resistant according to the recommendations of the Antibiogram Committee of the French Society of Microbiology (CASFM, 2022). The reference strain Escherichia coli ATCC 25922 was used as a control strain. Strains with resistance to a minimum of three to four antibiotics are considered multi-resistant (MDR: Multi-Drug Resistant).

## 2.2. Data Analysis

The data collected was coded, manually processed, processed, and analyzed using Office Excel 2007 and Epi Info version 7.2 software to generate descriptive statistics. Relative risks were calculated to control for confounding. Univariate and bivariate analyses were carried out to bring out the tables and figures with an *a* risk of 5% and a confidence interval of 95%, and a p-value < 5% was assumed

to be statistically significant.

## 3. Results

From January to December 2022, 93 strains of *Salmonella* were recorded out of 5480 samples analyzed at the NLCBPH, or 1.69%. The age of the patients ranged from 1 to 78 years with a mean of 26 years and the mode was 5 years with a standard deviation of 20.59. The 0 - 9 year old age group was 27% and the female gender was more representative at 53%, *i.e.* a male/female sex ratio of 0.89. **Figure 1** shows the frequency of *Salmonella* species isolated.

*Salmonella arizonae* strains were more represented with 52%.

**Figure 2** presents the frequency of *Salmonella* species isolated according to sample types.

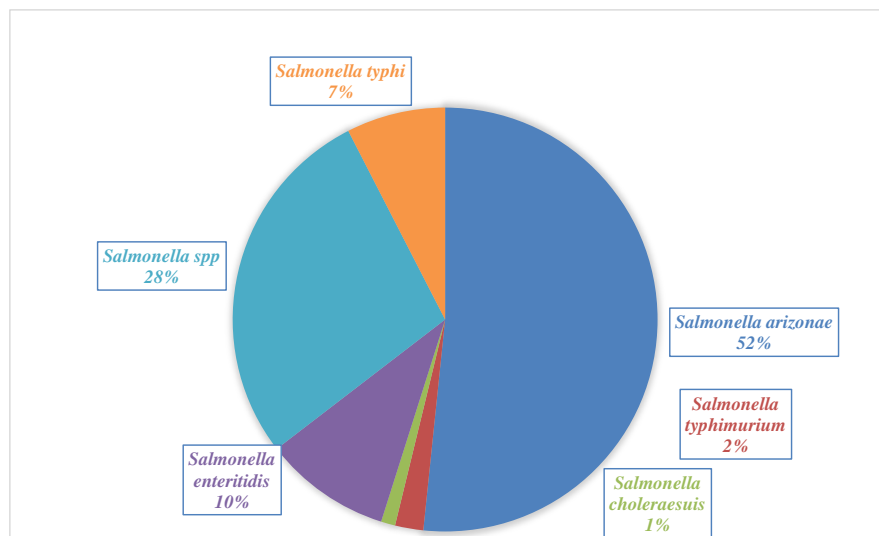*Salmonella* strains were more isolated from stool than from urine and blood.



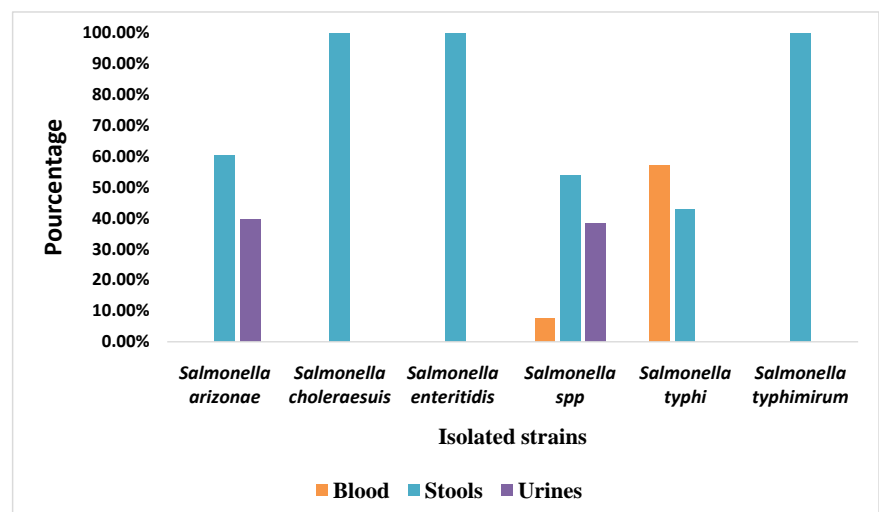**Figure 1.** Frequency of *Salmonella* species isolated.



**Figure 2.** Frequency of *Salmonella* species isolated according to sample types.

Table 1 presents the distribution of the antibiotic resistance rate of the isolated *Salmonella* strains.

*Salmonella* strains have a resistance rate to Tetracycline of 62.37% followed by Penicillins of 50.00%.

Figure 3 represents the rate of multi-antibiotic resistance of *Salmonella* strains.

*Salmonella* spp. strains were multi-resistant at 58.69%.

Table 2 presents the association between antibiotic resistance and *Salmonella* strains isolated.

Table 1. Distribution of the antibiotic resistance rate of *Salmonella* strains isolated.

| | *S. arizonae* N = 48 | *S. choleraesuis* N = 1 | *S. enteritidis* N = 9 | *S.* spp. N = 26 | *S. typhi* N = 7 | *S. typhimurium* N = 2 | Total N = 93 |
|---|---|---|---|---|---|---|---|
| **Antibiotics** | **%** | **%** | **%** | **%** | **%** | **%** | **%** |
| Definition | 8.33 | 0.00 | 22.22 | 7.69 | 14.29 | 50.00 | 10.75 |
| Ceftriaxone | 16.67 | 0.00 | 0.00 | 11.54 | 0.00 | 50.00 | 15.05 |
| Cefotaxime | 16.67 | 0.00 | 11.11 | 3.85 | 0.00 | 0.00 | 10.75 |
| Cefepime | 12.77 | 0.00 | 11.11 | 19.23 | 28.57 | 0.00 | 15.22 |
| **Cephalosporines** | **13.61** | **0.00** | **11.11** | **10.57** | **10.71** | **25.00** | **12.94** |
| Ciprofloxacin | 12.5 | 0.00 | 22.22 | 15.38 | 14.29 | 0.00 | 13.00 |
| Ofloxacin | 18.75 | 0.00 | 22.22 | 8.00 | 28.57 | 0.00 | 16.30 |
| Pefloxacin | 22.92 | 0.00 | 22.22 | 34.62 | 42.86 | 0.00 | 26.88 |
| **Fluroquinolones** | **24.55** | **0.00** | **25.92** | **19.33** | **28.57** | **0.00** | **21.59** |
| Amoxi + ac.clav. | 41.67 | 0.00 | 11.11 | 34.62 | 71.43 | 50.00 | 38.71 |
| Ampicillin | 70.83 | 0.00 | 22.22 | 61.54 | 71.43 | 0.00 | 61.29 |
| **Penicillin** | **56.25** | **0.00** | **16.66** | **48.08** | **71.43** | **16.66** | **50.00** |
| Amykacin | 14.58 | 0.00 | 0.00 | 23.08 | 0.00 | 50.00 | 14.05 |
| Kanamycin | 33.33 | 0.00 | 0.00 | 30.77 | 14.29 | 50.00 | 27.96 |
| Tobramycin | 25.00 | 100 | 11.11 | 7.69 | 0.00 | 0.00 | 17.20 |
| **Aminosides** | **24.30** | **33.33** | **3.70** | **31.51** | **4.76** | **33.33** | **21.82** |
| Chloramphenicol | 43.75 | 0.00 | 77.78 | 30.77 | 14.29 | 50.00 | 40.86 |
| Tetracyclin | 56.25 | 0.00 | 77.78 | 61.54 | 85.71 | 100.00 | 62.37 |
| Imipeneme | 8.33 | 0.00 | 0.00 | 3.85 | 0.00 | 0.00 | 5.38 |

Table 2. Association between antibiotic resistance and *Salmonella* strains isolated.

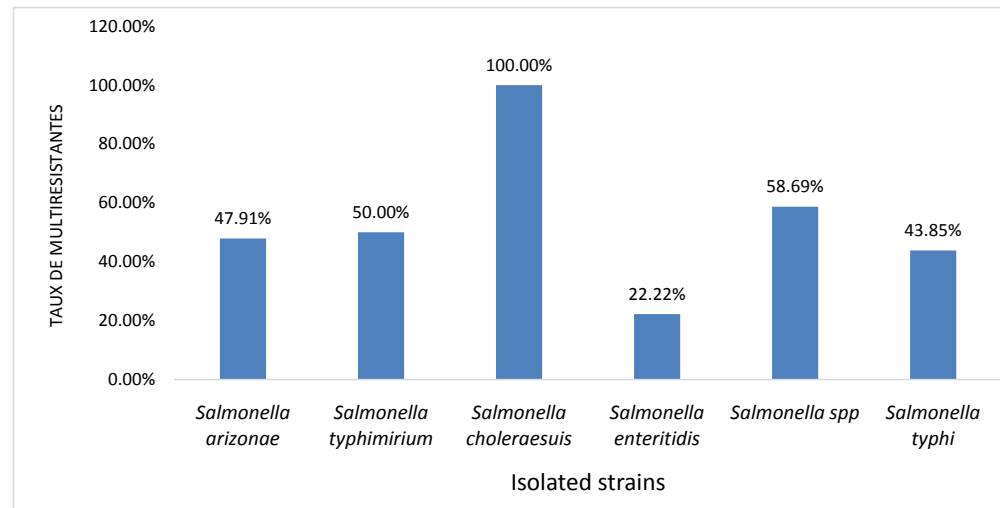| Antibiotics | N = 93 | % | RR | Chi² | IC (95%) | p-value |
|---|---|---|---|---|---|---|
| Cephalosporines | 12 | 12.90 | 0.38 | 15.95 | 0.22 - 0.66 | $6 \times 10^{-5}$ |
| Penicillins | 47 | 50.53 | 1.85 | 20.38 | 1.45 - 2.36 | $6 \times 10^{-6}$ |
| Fluoroquinolones | 18 | 19.35 | 0.59 | 6.42 | 0.38 - 0.91 | 0.001 |
| Aminosides | 21 | 22.58 | 0.70 | 3.26 | 0.47 - 1.05 | 0.007 |
| Chloramphenicol | 38 | 40.86 | 1.41 | 5.41 | 1.07 - 1.86 | 0.019 |
| Tetracycline | 58 | 62.36 | 2.46 | 51.68 | 1.99 - 3.05 | $10^{-10}$ |
| Imipenem | 5 | 5.37 | 0.15 | 32.44 | 0.065 - 0.36 | $10^{-10}$ |

**Figure 3.** Rate of multi-antibiotic resistance of isolated *Salmonella* strains.

There is a significant association between the different families of antibiotics and *Salmonella* strains ($p < 0.05$). Penicillins, phenols, and cyclins had a very high risk of being resistant to *Salmonella* strains (RR > 1).

## 4. Discussion

During the period from January to December 2022, 93 strains of *Salmonella* were isolated in stools, urines, and blood samples at the NLCBPH. Pre-enrichment was carried out to increase the sensitivity of the cultures. The number of isolates and their characteristics could differ depending on the media used and from one sample to another [23] [24].

The rate of *Salmonella* species isolated was 1.69%. This rate was below that obtained in the study carried out in Niger in 2015, by Langendorf *et al.*, who found a prevalence of *Salmonella* strains isolated from humans of 9.76% [25]. *Salmonella* spp. strains accounted for 28%. In the stools the rate was 53.85%, in the urines 38.46% and in the blood 7.69%. The results obtained in urines were similar (33.33%) to those of a study carried out by Brenda Kwambana-Adams *et al.* in Gambia from 2005-2015 [26]. This prevalence could be explained by the mode of transmission of *Salmonella* which could depend on dietary habits in each country. The fecal-oral mode of transmission would be one of the reasons for the high rate of contamination in stools.

*Salmonella typhi* strains accounted for 7%. The contamination rate in blood was 53.85% and 42.86% in stools. These results were superior to those of Mossoro-Kpinde *et al.* who reported in their study in 2012 in Bangui, a 50% rate of infection due to *Salmonella typhi* in blood culture [21]. *Salmonella typhi* strains pass into the blood after the incubation period.

In the stools, *Salmonella enteritidis* strains represented 10%, *Salmonella typhimurium* 2%, and *Salmonella cholestasis* 1%. Contrary to the results of work carried out in the Democratic Republic of Congo in 2010, by Vandenberg *et al.*,

who isolated 61% of *Salmonella typhimurium* and 22% of *Salmonella enteritidis* in the blood [27]. This difference would be due to the size of our sample and the study period. The way of eating, the anarchic and uncontrolled opening of tourniquets, and the sale of frozen foods in the CAR are believed to be the cause of the contamination and multiplication of strains of *Salmonella enteritidis* and *Salmonella typhimurium*.

The 0 - 9 year old age group was the most represented with a *Salmonella* contamination rate of 29%. This result is similar to that of Kariuki *et al.* in 2006 in Kenya who isolated 336 strains of non-typhoid *Salmonella* in children aged 0 to 13 years in his study [28]. The age group > 50 years old had a rate of contamination with *Salmonella* strains of 11%. This result was below the study conducted by Delmas *et al.* in 2010, in France which obtained 14% in age groups > 65 years [29]. Elderly patients respect hygiene rules, which could explain a relatively lower contamination rate in both countries. The multivariate analysis showed no association between age groups and antibiotic resistance of the *Salmonella* strains isolated (p > 0.05).

The female sex was more represented with 53% compared to 47% of the male sex, *i.e.* a male/female sex ratio of 0.89. This result is very close to that reported by Salim *et al.* in 2017 in Algeria who reported in their study a female predominance of 66.66% [30]. The national *Salmonella* reference center in Brussels, Belgium in 2022, showed that there was no significant difference in the distribution between men and women [31]. The multivariate analysis showed no association between sex and the rate of antibiotic resistance of the *Salmonella* strains isolated (p > 0.05).

The rate of resistance to Chloramphenicol was 38% among *Salmonella arizonae* strains in this study. In contrast, a study carried out by Mossoro-Kpinde *et al.* in 2012, in Bangui revealed no resistance to Chloramphenicol [21]. This difference could be explained by the fact that Chloramphenicol was considered a first-line antibiotic for the treatment of salmonellosis which, nowadays, is replaced by 3rd generation Fluroquinolones and Cephalosporin [32].

The resistance rate to Ciprofloxacin was 15.38%, to Cephalosporins 10.57% and Tetracycline 61.54% on strains of *Salmonella* spp. Compared to the resistance rate of a study carried out by Sawadogo Néhémie in 2022, in Bamako which obtained a resistance rate to Ciprofloxacin of 20% [33]. According to the report from the national *Salmonella* reference center in Brussels, Belgium in 2022, resistance to Ciprofloxacin had increased after 4 years from 11% in 2021 to 17% in 2022 [31]. Our results were superior to those of a study carried out by Sounkarou in Mali in 2013, which obtained 2.5% resistance to cephalosporins [34]. This high rate shows an emergence of resistance to cephalosporins in strains of *Salmonella* spp. in patients who consume this antibiotic inappropriately. In another study conducted by Andoh *et al.* in 2016 Ghana had a high rate of resistance to Tetracycline 80.7% [35]. The uncontrolled use of antibiotics and especially the ease of finding Tetracycline at a low cost contribute to the resistance of

this antibiotic on strains of *Salmonella*.

*Salmonella enteritidis* strains had a Chloramphenicol resistance rate of 77.78%. A study carried out in The Gambia from 2005 to 2015 by Brenda Kwambana-Adams *et al.* had shown resistance to Chloramphenicol in 41 strains of *Salmonella* enteritidis [26].

*Salmonella typhi* strains had a resistance rate to Ciprofloxacin of 14.29%, to Tetracycline of 85.71%, and Ampicillin and Amoxicillin/clavulanic acid of 71.43% each. These results are similar to those of Mossoro-Kpinde *et al.* who found in their study in 2012, in Bangui, a rate of resistance to Ciprofloxacin of 7%, to Tetracycline 16.3%, to Ampicillin 72.1% and to Amoxicillin/clavulanic acid 25.6% [21]. These results were statistically significant between the resistance rates to the different antibiotics used and to the *Salmonella* strains with $p < 0.05$.

The rate of multi-antibiotic resistance of *Salmonella* strains was 48.38%. The rate of multi-antibiotic resistance of *Salmonella* spp. strains was 58.69%. These results were superior to those of the study conducted by Abba *et al.* in 2017 in Chad on strains of *Salmonella* spp., which had a rate of multi-resistance to antibiotics of 34.1% [36]. Previous studies by Novick *et al.* in 1981 showed that the uncontrolled use of antibiotics in poultry production has intensified the emergence of multi-resistant bacteria [37]. An analysis carried out by Kasper *et al.* in Cambodia in 2018 on 41 cases of typhoid fever showed a rate of multi-resistance to antibiotics of 56% [38]. The high-level rate of multi-resistance to antibiotics would be due to strains originating from the environment where antibiotics are in daily use [39].

## 5. Conclusion

The strains of *Salmonella* isolated in the different samples represent a zoonotic health danger, constitute a public health problem and still remain a current subject in the Central African Republic. According to the results obtained in this study, Penicillins, Phoenics, and Cyclins had a high rate of resistance to *Salmonella* strains. It should be noted that several factors favor the emergence of antibiotic resistance, including socioeconomic conditions, the lack of implementation of basic hygiene rules, and especially the uncontrolled use of these antibiotics by patients. The study showed that antibiotics that were effective against *Salmonella* strains are becoming increasingly resistant. These data can serve as a basis for setting up a program to collect epidemiological data for phenotypic and genotypic detection of antibiotic resistance factors. The study of the genetic diversity of strains, as well as the monitoring and control of antibiotic resistance in *Salmonella* strains, is necessary for the Central African Republic, especially since multi-antibiotic resistant strains had a high rate in this study.

## Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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