Original Research Article

Prevalence and risk factors of *Salmonella spp* and *Escherichia coli* multi-resistant to antibiotics in village poultry farming in the commune of Doba in Chad.

ABSTRACT

Introduction: Avian colibacillosis and salmonellosis are ~~pathologies~~ diseases that have a major economic impact on poultry farming and public health worldwide. This study aims to determine the prevalence ~~of the risk factors~~ and associated risk factors of multidrug resistant Salmonellosis and *Escherichia coli* in Doba commune, Chad ~~multiresistant to antibiotics.~~

Methodology: This is an experimental study that took place in Doba, the survey of which was carried out among 41 poultry farming households in 15 districts. Feces were collected and cultured. The strains isolated were identified by the Enterosystem 18R gallery. Their antibiotic sensitivities were tested against 12 selected antibiotics. The Epi Info 7 ™ software was used to perform the statistical analyses.

Results: For this study, 193 samples of droppings were cultured. However, 24 (12.44%) strains were isolated and identified, including 13 (6.74%) *Salmonella spp* and 11 (5.70%) *Escherichia coli*. These strains of Salmonella spp were more sensitive to Imipenem (92.31%). They were more resistant to Clotrimazole (84.62%). For *E. coli*, the highest sensitivity was observed in Imipenem (76.92%). It was more resistant to Amoxicillin + Clavunalic Acid (AMC) 69.23%. Non-compliance with food hygiene, lack of maintenance of

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Comment [FG3]: How many samples were taken in each district?

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the habitat, non-compliance with prophylactic and sanitary measures were the risk factors most linked to the presence of salmonellosis and *E. coli*. According to the profile, the resistance of *Salmonella spp* to antibiotics was more observed in Béraba (23.08%) and that of *Escherichia coli* in Bédokassa (27.27%).

Conclusion: Non-compliance with biosecurity measures and the uncontrolled use of antibiotics have favored the emergence of Salmonella spp and Escherichia coli multiresistant to antibiotics.

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Keywords: Prevalence, *Salmonella spp*, *Escherichia coli*, multi resistant, antibiotics, Doba/Chad.

1. INTRODUCTION

Avian salmonellosis is a cosmopolitan disease with a variable distribution of serotypes. Salmonella are ubiquitous bacteria, very widely established in poultry farms, so that their elimination requires the implementation of considerable means. Salmonella are one of the first causes of collective food poisoning (TIAC) **[1]**. This pathology constitutes a serious obstacle to the development of the poultry industry, especially in the developing countries of Africa and Asia. It can be the cause of high mortality rates in farms, thus leading to significant economic losses **[2]**. Salmonella is a gram-negative pathogen, causing a wild range of illnesses including typhoid fever, bacteraemia, intestinal and systemic catarrh **[3]**. Among the 2600 known serovars of Salmonella, some serovars are only pathogenic for certain animals. For example, *Salmonella enterica* serovars *Gallinarum biovar Pullorum* (*S. Pullorum*) specifically infects poultry, causing “white diarrhoea” (Pullorum disease) and avian typhoid, respectively, followed by a high mortality rate in 20-day-old chickens **[4, 5, 6, 7]**. *Salmonella gallinarum* can spread to reproductive organs, resulting in vertical transmission of the pathogen, as well as egg-related salmonellosis. Thus, the detection of S. Pullorum/Gallinarum is very important because poultry products (chickens and eggs) intended for human consumption are reservoirs of Salmonella **[8, 9]**. Although some

infections come directly from domestic animals, reptiles or contaminated water, the percentage of transmission through food is estimated at 95% **[10]**. Colibacillosis and avian salmonellosis are bacterial pathologies that cause serious pathological problems such as growth retardation and mortalities in the poultry industry on the one hand and economic problems related to control measures on the other **[11, 12]**. Poultry farming in Chad is family-based and is practiced by 90% of households. Its exploitation contributes to meeting food needs (particularly in quality animal protein), improving household income for the acquisition of everyday consumer goods and strengthening social ties. Thus, this sector contributes to the fight against poverty and food insecurity. But the interest given to the protection of poultry varies according to the social situation **[13]**. Avian pathologies are the main obstacles to the development of this sector. Lack of mastery of poultry techniques, ignorance of biosecurity measures and risk factors, and the anarchic and non-conventional use of veterinary drugs favor the appearance of avian pathologies and the resistance of pathogens. In Chad, very few data are available on antibiotic resistance linked to the consumption of proteins of animal origin. Nor is there a surveillance network for bacterial antibiotic resistance **[14]**.

This research work carried out in the city of Doba aims to determine the prevalence of strains of Salmonellosis and *Escherichia coli* multi-resistant to antibiotics and the risk factors associated with these pathologies in village poultry farming in the Municipality of Doba.

2. MATERIAL AND METHODS

2.1. Study frameworks

The Doba Provincial Hospital allowed the samples to be stored at +4°C. The samples were processed in the laboratory of the Livestock Research Institute for Development (IRED) in N'Djamena.

2.2. Poultry household survey

In order to prepare the poultry farmers for the samples on the one hand and to know the conditions of poultry breeding in the Commune of Doba on the other hand, an interview was

carried out in forty-one (41) households, distributed in fifteen (15) districts which are: BédogoI, Bédogo II, Bédogo III, Béraba, Bédokassa, Cotontchad, Djarabé I, Djarabé II, Miscellaneous, Dobaya, Tembi, Yeldanem, Maihongo, Takasnan and Gaki.

The survey took place during the period from April to July 2022 and allowed poultry farmers to learn more about the importance given by research to animal and human health.

2.3. Poultry Health Information

For animal health research, sick poultry were treated with certain broad-spectrum antibiotics and antiparasitics such as oxytetracycline, amoxicillin, amprolium and albendazole.

2.4. Sample collection

Cloacal swabs were performed with the help of a technician who was in charge of holding the chicken and keeping it in a transverse position in order to facilitate the operation. For health prophylaxis reasons, swabbing was done at the entrance to the henhouse early in the morning and late in the evening when the chickens were in the henhouse.

The samples taken were fresh droppings taken using swabs introduced into the rectum. Once the swab is removed from the rectum, it is inserted into the numbered tube containing Cary Blair's transport medium and broken halfway into the tube and the tube is resealed and returned to the cooler with the fresh packs. Samples are taken from live chickens. The samples are stored at +4°C in the refrigerator at the Doba Provincial Hospital and then transported by bus service to the IRED laboratory in N'Djamena.

2.5. Selective enrichment of samples and their culturing

We used Buffered Peptone Water (EPT) and Rappaport-Vassilladis-Soja (RVS) for selective enrichment of the samples. Eosin Methylene Blue (EMB), Xylose-Lysine-Deoxycholate (XLD), Salmonella-Shigella (SS) and Mueller Hinton agars were used to culture samples and isolate strains.

2.6. Identification of *Salmonella* and *E.coli* strains

The different species are identified from the Enterosystem 18R gallery. The principle, the preparation of the gallery, the preparation of the inoculum, the inoculation of the gallery, the

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reading and the identification of the species were respected in accordance with the manufacturer's protocol.

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2.7. Antibiotic strain susceptibility testing

The choice of antibiotics is made according to the recommendation of the Antibiogram Committee of the French Society of Microbiology (CA-SFM 2020). Twelve (12) antibiotics from the first and second groups were chosen to test the sensitivity of these strains. These are: Imipenem (IPM), Amoxicillin (AMX), Cefuroxime (CXM), Clotrimazole (CLT), Ceftazidime (CAZ), Aztreonam (ATM), Ciprofloxacin (CIP), Cotrimoxazole (SMX5), Amoxicillin + Clavunalic acid (AMC), Gentamicin (GM), Cotrimoxazole (SMX25), Nalidixic Acid (NA) **[15]**.

2.8. Statistical analyzes

Data were entered into Microsoft Excel 2007 software and transferred to Epi Info 7™ software for statistical analyses. All percentages are preceded by their absolute values. The results are presented in the form of tables and figures.

3. RESULTS

3.1. Number of village poultry farms surveyed and samples taken

A total of 41 households were randomly identified following visits made for information and awareness campaigns. Five (5) samples were taken per household from which 205 samples were taken. However, twelve (12) or 5.85% were eliminated for contamination.

3.2. Characteristics of information from poultry farmers

The surveys carried out on households by means of a questionnaire provided a certain amount of information, the details of which are presented in Table 1.

Table 1: Summary of the characteristics of the questionnaires asked to poultry farmers

	Total N=41 (%)	
Settings	**Yes**	**No**
Sanitary barrier	11 (26.83)	30 (73.17)
Food hygiene	0	41 (100)

Antibiotic treatment		18 (43.90)	23 (56.10)
Technician follow-up		0	41 (100)
Technical knowledge		13 (31.71)	28 (68.29)
Litter		0	41 (100)
	Clean		
Environment		27 (65.85)	14 (34.15)
	Dirty	14 (34.15)	27 (65.85)
Natural food	Raw	37 (90.24)	4 (9.76)
	Provend	4 (9.76)	37 (90.24)
Source of drinking water	Well	24 (58.54)	17 (41.46)
	Faucet	17 (41.46)	24 (58.54)
Chicken coop floor	Earthbeaten	39 (95.12)	02 (4.88)
	Cemented	02 (4.88)	39 (95.12)

3.3. Prevalence of isolated strains

A total of 193 samples of droppings collected from barnyards were cultured. However, 24 (12.44%) strains were isolated and identified by the Enterosystem 18R gallery, including 13 (6.74%) *Salmonella spp* and 11 (5.70%) *Escherichia coli*.

3.4. Risk factors for *Salmonella spp* and *Escherichia coli*

Among the data recorded using the questionnaire, we identified factors associated with the likelihood of poultry being positive for *Salmonella spp* and *Escherichia coli*. We found that non-compliance with food hygiene, lack of quarantine and lack of litter were the highest risk factors, details can be found in Table 2

Table 2: Factors behind the risk of appearance of *Salmonella spp* and *E. coli*.

Risk factors	**Total N=41 (%)**
Non-compliance with food hygiene	41 (100)
Non-compliance with watering hygiene	5 (12.20)
Non-respect of sanitary barrier	25 (60.98)
No quarantine	41 (100)

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Lack of litter	41 (100)
Unhealthy food storage place (store)	13 (31.71)
Unclean environment	21 (51.22)
Traditional farming system	37 (90.24)
Water source (well)	24(58.54)

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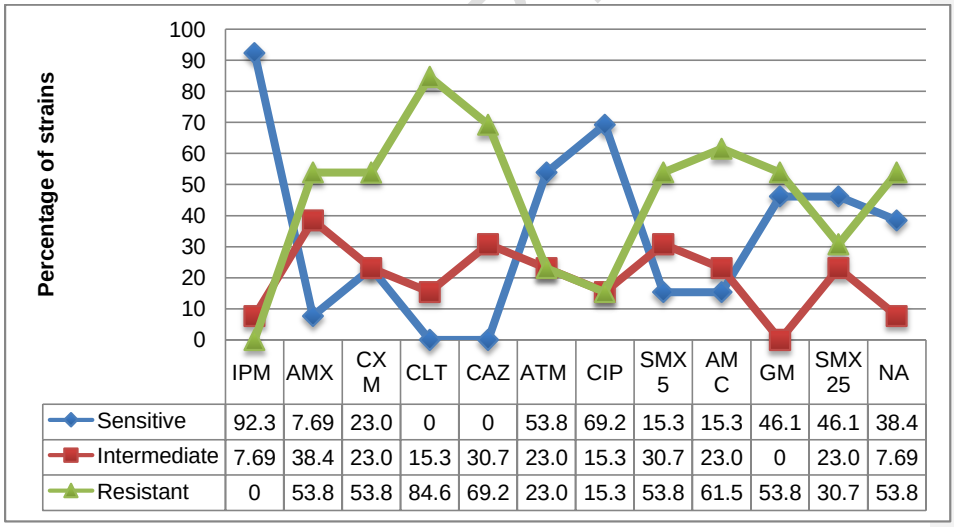
3.5. Prevalence of sensitivity and multi-resistance of *Salmonella spp* to the antibiotics tested

The susceptibility of 13 strains of *Salmonella spp* was tested to 12 antibiotics (IPM, AMX, CXM, CLT, CAZ, ATM, CIP, SMX 5, AMC, GM, SMX 25, NA). FIG. 1 illustrates for each antibiotic tested the SIR profiles [Sensitive (S), Intermediate (I) and Resistant (R)] expressed as a percentage of the strains.

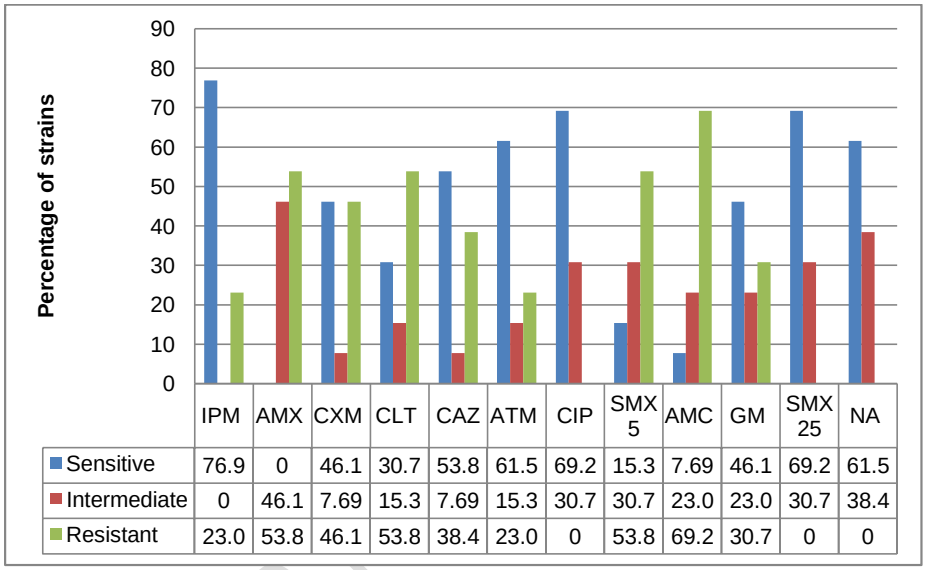


	IPM	AMX	CXM	CLT	CAZ	ATM	CIP	SMX 5	AMC	GM	SMX 25	NA
Sensitive	92.3	7.69	23.0	0	0	53.8	69.2	15.3	15.3	46.1	46.1	38.4
Intermediate	7.69	38.4	23.0	15.3	30.7	23.0	15.3	30.7	23.0	0	23.0	7.69
Resistant	0	53.8	53.8	84.6	69.2	23.0	15.3	53.8	61.5	53.8	30.7	53.8

Figure 1: SIR profiles of *Salmonella spp* strains tested with twelve (12) antibiotics

3.6. Prevalence of sensitivity and multi-resistance of *Escherichia coli* to the antibiotics tested

The 11 strains of *Escherichia coli* were also tested with 12 antibiotics (IPM, AMX, CXM, CLT, CAZ, ATM, CIP, SMX 5, AMC, GM, SMX 25, NA). FIG. 2 presents for each antibiotic tested the SIR profiles [Sensitive (S), Intermediate (I) and Resistant (R)] expressed as a percentage of the strains.



	IPM	AMX	CXM	CLT	CAZ	ATM	CIP	SMX 5	AMC	GM	SMX 25	NA
Sensitive	76.9	0	46.1	30.7	53.8	61.5	69.2	15.3	7.69	46.1	69.2	61.5
Intermediate	0	46.1	7.69	15.3	7.69	15.3	30.7	30.7	23.0	23.0	30.7	38.4
Resistant	23.0	53.8	46.1	53.8	38.4	23.0	0	53.8	69.2	30.7	0	0

Figure 2: SIR profiles of *Escherichia coli* strains tested with twelve (12) antibiotics

3.7. Distribution by district of strains of *Salmonella spp* and *Escherichia coli* resistant or with intermediate resistance to antibiotics in the city of Doba.

The 13 strains of *Salmonella spp* and the 11 of *Escherichia coli* resistant or with intermediate resistance were distributed by district. In order of importance of frequency according to the profile, the resistance of *Salmonella spp* to antibiotics was more observed in Béraba (23.08%) and that of *Escherichia coli* in Bédokassa (27.27%) and Yeldanem (18.18%). %). For details see Table 3

Table 3: Profile of strains of Salmonella spp and Escherichia coli resistant or intermediate resistance by district.

Neighborhoods	***Salmonella spp*** **N=13 (%)**		***Escherichia coli*** **N=11 (%)**	
	Intermediate	resistant	Intermediate	resistant
Béraba	1 (7.69)	03 (23.08)	-	-
Cotontchad	-	01 (7.69)	-	01 (9.09)
Djarabé 3	-	01 (7.69)	-	-
Divers	1 (7.69)	01 (7.69)	-	-
Maihongo	-	01 (7.69)	-	-
Gaki	-	-	1 (9.09)	01 (9.09)
Bédokassa	-	-	-	03 (27.27)
Takasnan	-	01 (7.69)	-	01 (9.09)
Tembi	-	01 (7.69)	-	01 (9.09)
Yeldanem	-	02 (15.38)	1 (9.09)	02 (18.18)
Total	2 (15.38)	11 (84.59)	2 (18.18)	9 (81.81)

4. DISCUSSION

Comment [FG14]: There is no discussion on the topic of associated risk factors

Prevalence of sample contamination by *Salmollaspp*

The results of bacteriological analyzes showed that 6.74% of the samples of faeces taken from the cloaca of local breed chickens were contaminated with *Salmonella spp*. This contamination may be the consequence of poor breeding practices by the poultry farmers surveyed. This rate is higher than that of Butayeand his team in 2006 which is 3.67% obtained in France **[16]**. According to these authors, regarding *Salmonella* contamination of chickens, non-typhoidal *Salmonella* are widely disseminated in nature, colonizing a wide range of animals including mammals, amphibians, reptiles, birds and insects. This

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observation is similar to our results where poultry cohabit with insects, rodents and wander in nature.

Other studies carried out in Chad have presented rates higher than ours ~~representing respectively~~at 65.85%, 38.46% and 43.75% respectively **[17, 18, 19]**. This low rate obtained in our study is justified by the fact that several common germs were isolated and eliminated and the number of farms surveyed is not representative of farms in the Municipality of Doba.

According to BODERING**[18]**, this fairly high prevalence reflects contamination of broiler chickens in N'Djamena and traditional ones in Doba. This high contamination is justified by the fact that more than half of the farms have insufficient prophylaxis and poor hygiene practices in buildings and livestock equipment.

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For Hamadou's study, 50% of breeding sites do not have a well-defined health plan and 85.7% do not have isolation measures. In addition, the relatively high rate of prevalence could be explained by several factors such as: the size of the sample which is not representative of all the sites of N'Djamena, non-compliance with the conditions of hygiene, the absence of health surveillance programs and the non-compliant prophylactic use of antibiotics against *Salmonella***[17]**.

In Tabo's study, this permanent excretion of germs (Salmonella) by poultry would be due to stress factors such as high temperatures, hygrometric deficits, the beginning and end of the laying period, etc.) **[19]**.

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Prevalence of sample contamination by *Escherichia coli*

In the 193 samples of feces taken from the cloaca of local breed chickens and placed in culture, *Escherichia coli* was isolated at a rate of 5.70%. This contamination is linked to poor farming practices. The environment in which the farms are conducted is favorable to the emergence of strains because chickens roam everywhere in nature to feed and drink.

A study carried out in France showed a similar rate of 5.96% **[20]**. These authors justified this contamination by the fact that *Escherichia coli* is part of the commensal flora of the

digestive tract of birds and poultry can be contaminated by various sources (birds, rodents, insects, wild birds, water, dust, environment).

This rate is lower than that of BOUBA **[12]**, in Ngaoundéré in Cameroon with a rate of 100%. According to this author, *Escherichia coli* is a ubiquitous bacterium in the environment whose contamination is very high in poultry farming. Although all farms have a prophylaxis program that they follow, 84.2% of these medical prophylaxis programs do not take avian colibacillosis into account. Rats and mice are important sources of contamination for poultry **[21]**. However, in this study, mice and rats are present in 100% of the farms, which represents a considerable risk.

This rate is also lower than the 19.23% obtained in the study carried out in Chad **[18]**. According to these authors, this high contamination is justified by the fact that more than half of the farms have insufficient prophylaxis and poor hygiene practices in buildings and livestock equipment. According to Lutful**[22]**, *E. coli* is the largest bacterial population in the digestive tract of poultry because of the 109 CFU of bacteria in 1 g of poultry faeces, 106 are *E. coli*.

Prevalence of susceptibility of Salmonella spp strains to antibiotics

Based on the analyzes of this study, salmonellosis can be considered an important poultry disease. The results showed that no strain of *Salmonella spp* isolated is sensitive to Clotrimazole and Ceftazidime, however, 92.31% of the strains are sensitive to Imipenem, 69.24% to Ciprofloxacin and 53.84% to Aztreonam.

This strain susceptibility rate of 69.24% obtained for Ciprofloxacin is lower than that of 100% in another study in Chad **[17]**, while the rate of 92.31% obtained for Imipenem is far above the results. According to these authors, fluoroquinolones are used very little or not at all in the poultry could sector in Chad and this justify this sensitivity of the strains.

Multi-resistance of *Salmonella spp* strains to antibiotics

In our study, the prevalence of ~~strain~~ *E.coli* which wereresistance to amoxicillin was 53.85%. This rate is very high compared to that of 7.32% obtained for this same molecule in Chad

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[17]. This resistance of the strains to antibiotics is justified by the fact that 100% of the farms surveyed are not monitored by a technician and that the breeders treat the poultry themselves, mainly using tetracycline as a preventive and curative measure associated with traditional treatments. Also, a few breeders have had their poultry vaccinated.

For Ciprofloxacin, 15.25% of the strains isolated were resistant. In addition, the study also showed 100% resistance of these strains to Ampicillin and Imipenem. Surveys of poultry farmers reveal that 71.43% of breeding sites do not vaccinate their poultry and 57.15% use antibiotics arbitrarily for treatment.

The multiresistance of the *Salmonella spp* strains isolated in this study varies from 3 to 7 antibiotics. Resistance to these families of antibiotics is also quite low in France with rates close to 1% **[23]**. These differences could find their explanation in the habits of consumption of antibiotics which differ according to the regions of the world **[11]**. Several authors have also observed that the support for resistance in poultry was plasmidic **[24, 25]**.

For Gentamicin, a rate of 53.85% is obtained in our study while no strain ~~is resistant~~were found to be resistant in the study carried out by Hamadou and his team in 2017.

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Prevalence of susceptibility to *E. coli* to antibiotics

The results of the study showed that 76.92% of the strains isolated were sensitive to Imipenem; 69.23% with Ciprofloxacin and Cotrimoxazole (SMX25); 53.85% to Ceftazidime; 61.54% Nalidixic Acid and Aztreonam. On the other hand, the results showed that no isolated strain is sensitive to amoxicillin. This result is similar to that of BOUBAin 2014 and Hamadou in2017.

According to BOUBA **[12]**, in the context of this study where 73.7% of farms do not benefit from any veterinary follow-up, the possibility that breeders use antibiotics inappropriately and abusively exists and could be the cause of the high percentage of multi-resistant strains (97.06%) observed. Indeed, there is a close relationship between the use of antibiotics and the appearance of bacteria resistant to said antibiotics.

For Hamadou**[17]**, the strains studied resist these antibiotics by producing beta-lactamases which inactivate them.

Multi-resistance of *E. coli* to antibiotics tested

In this study, the strains were 53.85% resistant to Clotrimazoles. A study carried out in Casablanca, Morocco presented resistance rates of 88.23% and 50.20% to amoxicillin and nalidixic acid **[26]**. This high resistance to antibiotics in this country is due to the expansion of production that took place without the systematic control of hygiene throughout this sector. Regarding the resistance of *E. coli* to gentamicin we obtained a rate of 30.77% which is higher than that of 3.7% obtained in Rwanda **[27]**. We can say that the abusive and long-lasting use of antibiotics has led to the emergence of bacteria resistant to these drugs.

A study carried out as part of antibiotic resistance monitoring by NSES in 2021 **[28]** revealed that most strains that show resistance to at least 3 antibiotics are predominant in turkeys (38.6 %) and in chicken (38.3%). This rate is lower than the rate of 53.85% obtained in our study. According to this study, even if resistance is a natural phenomenon of bacterial defense, the intensive use of antibiotics or their misuse are accelerators of this phenomenon. Moreover, these resistances can spread, both between bacteria of the same species and in bacterial populations belonging to different bacterial genera, by horizontal transfer of resistance genes. This type of transfer is not limited to commensal or pathogenic bacteria of humans or those of animals, but can occur between these two compartments and the surrounding ecosystems. As humans and animals can share the same bacteria and sometimes the same therapeutic arsenal, the use of antibiotics in one of the compartments can thus lead to the appearance of resistance in the other compartment.

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5. CONCLUSION

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This study ~~allowed the identification~~ has successfully identified/reported the prevalence of *Salmonella sp* (6.74%) and *Escherichia coli* (5.70%) which is worrying for the health of poultry. It also allowed us to understand poultry farming practices in households and to

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realize that Salmonellosis and Colibacillosis are pathologies that circulate within family farms. These two pathologies are the consequences of poor husbandry practices. These bad practices with the inappropriate use of antibiotics have favored the emergence of multi-resistant germs in these farms.

However, supervision and awareness-raising work for poultry farmers must continue to contribute to the improvement of family poultry farming in the Municipality of Doba.

Comment [FG23]: Sentence is confusing and should be rewrite

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Comment [FG24]: Your reference format is improper and not according to guidlines

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