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# Serotypes and Multiresistant *Salmonella* sp. from Chicken Eggs and Laying Hens in Burkina Faso

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**Abstract** : The study investigated the prevalence and antibioresistance profil of *Salmonella* sp. serovars isolated from eggs and poultry in stock farming. A total of 245 eggs and 98 laying hens fecal matters from different breeding sheds were collected. The samples were processed for identification and serotyping of *Salmonella* sp using microbiological standard methods and Kauffman-White scheme respectively. *Salmonella* sp isolates antibiotic susceptibility to antimicrobial agents was tested by disk diffusion method. A total of 63 *Salmonella* isolates were recovered with positive samples from eggs (11.8%) and from faecal matter (12.24%). The successful serotyping of 53/63 isolates revealed the presence of *S.* Typhimurium (11.11%), *S.* Kentucky (1.59%), *S.* Ouakam (1.59%), *S.* Brancaster (6.35%), *S* Hato (6.35%), *S.* Essen (3.17%), *S.* Cannstatt (1.59%), and *S.* Derby (36.51%). Ten strains (15.87%) were untypable and ten (15.87%) belong to different serogroups such F and O. All the sérotypes shown resistance to at least one antibiotic while, 41 (65.08%) were multi-resistant to Erythromycin, Streptomycin, Tetracycline, Ceftriaxon, while high sensitivity was recorded for Chloramphenicol, Ciprofloxacin, Nalidixic acid, Imipenem, Cephalexin, Sulfamethoxazole–trimethoprim and Colistin Sulfate. These results suggest that eggs from stock farming are contaminated and harbour resistant *Salmonella* sp. It highlights worry in antibiotics use in stock farming, the need for farm workers and consumers education about safe handling of eggs.

**Keywords:** *Salmonella* Serotypes, Antimicrobial Resistance, Eggs, Layer Hens, Burkina Faso

## Introduction

*Salmonella* is one of the major causes of foodborne disease outbreaks (Naik *et al.*, 2015; Feasey *et al.*, 2012; Sharkawy *et al.*, 2017). Among the most important microorganisms, *Salmonella* spp. may be considered one of the most circulating and frequent foodborne agents in the world (CDC, 2016; EFSA, 2017) and may cause significant damage to the poultry industry as well as to public health. Importantly, products of avian origin represent 47% of salmonellosis sources in humans (CDC, 2016). In poultry products *Salmonella* spp. was the main cause of early warnings in the developed countries these last years (RASFF, 2018).

*Salmonella* spp control is a major concern for poultry producers in several countries (Fonseca *et al*., 2019). It is possible that this bacterium remains in the farm environment as a biofilm form. *Salmonella* sp biofilms in eggs is able to enter the egg into albumen and yolk one day of contact with eggs hells (Barrow and Lovell 1991, Gustin, 2003; Fonseca *et al*., 2019). Chicken and eggs in particular continue to be identified as important sources for human Salmonellosis (Van Schothorst and Notermans, 1980; Tauxe, 1996; Thong *et al.*, 2002, Finstad *et al.*, 2010; Mead *et al*, 2010). However in developing country the scarce data are available on the role of poultry and product in *Salmonella* sp epidemiology because of the lack of epidemiological surveillance systems


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(Ejeta *et al.*, 2004; Steven *et al.*, 2006). Poultry farming system in developing country are divers from free roaming poultry without veterinary care to modern exploitation with veterinary care (Kagambega *et al.*, 2012; 2013). Therefore, distribution of *Salmonella* serotypes from poultry sources is geographically variable and changes over time, although several serotypes are consistently detected at a high incidence (Davies *et al.*, 2001; Cardinale *et al*., 2003, 2004; Kagambega *et al*., 2013). Many of the *Salmonella* serotypes that are most prevalent in humans are also common in poultry (Van Duijkeren *et al.*, 2001). The emergence of antimicrobial resistant *Salmonella* is mostly associated with the non-therapeutic use of various classes of antimicrobials in large quantities in food animals (Marshall and Levy, 2011; Mir *et al.*, 2015; Wales and Davies, 2015). Therefore, this study was undertaken to characterize antimicrobial resistance of *Salmonella* spp strains occur in farm and chicken eggs carriage.

## Materials and Methods

### Farm environment and eggs samples collection

The assessment stocks farming breeding sheds soil contamination was carried out by using aseptically sterile overshoes to walk and trap laying hens fresh defecated matter. The pair of over shoes was aseptically transferred into sterile container. Thus a total of 98 samples were collected in Ouagadougou and Bobo Dioulasso from February to September. Then, a total of 245 egg samples were collected concomitancy during visiting of stocks farming. All samples were transported to the laboratory for cultivation and isolation within the 24 hours.

### *Salmonella* isolation

Samples were processed for *Salmonella* isolation and identification according to the International Organization for Standardization norm 6579-2017.

For the faecal matter, each over overshoes was homogenized into sterile buffered peptone water to 9/10 (w/v).

A pool of 5 eggs constituted one sample analysed. The outer surface carefully were aseptically washed in a sterile bag containing 225 ml of buffered peptone water (BPW), then remove the eggs one by one and rinsed in a 70% alcohol and placed on absorbent paper to removed excess alcohol.

The alcohol rinsed eggs the 5 eggs per samples were are broken, the content (yolk and albumen) collected in a sterile bag and mix genteelly 15 seconds at room temperature. For Pre-enrichment 50 ml of the mixture were homogenised in 200 ml buffered peptone water preheated to 35°C, then adjusted to 500 ml with BPW and shake genteelly.

After pre-enrichment at 37°C for 24 hours, 1 mL of each pre-enriched sample was enriched into 10 mL of Muller-Kauffmann broth novobiocin tetrathionate (MKTTn) (OXOID, England) at 37°C for 24 hours. After that, 0.1 ml of an enriched sample was transferred into 10 ml MSRV agar (modified semi-solid agar medium of Rappaport- Vassiliadis) (OXOID, England) and incubated at 42°C for additional 24 hours before plating a loop full on Xylose Lysine tergitol 4 (XLT4) and Xylose Lysine desoxycholate (XLD) incubated at 37°C for 24 hours.

The suspected *Salmonella* were confirmed biochemically by catalase and peroxidase production, the oxidation/fermentation tests, production of indol and $H_2S$, and fermentation of glucose, lactose and urea (Quinn *et al.*, 2011) and the API 20E (Biomerieux, Marcy l'Etoile, France). The strains were stored in Broth brain heart supplemented with 30% of glycerol at -20°C for further characterization.

### Serotyping

The colonies confirmed as *Salmonella* spp. were serotyped according to the White-Kauffmann-Le Minor scheme described by Popoff *et al.,* (2004). Serotyping was performed at the laboratory Anses, Hygiene and Quality of Poultry and Pig Products Unit, Plouflagan, France.

### Antimicrobial susceptibility testing

The antimicrobial susceptibility tests were performed on Mueller Hinton agar using the disk diffusion method (Bauer *et al.,* 1966). Interpretation of MICs and zone diameters was done according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST 2017). The antimicrobials tested were gentamicin (GEN; 10 µg), Streptomycin (STR; 10 µg), Aztreonam (AZT; 30 µg), Ticarcillin (TC; 75 µg), Imipenem (IPM; 10 µg), Amoxicillin-clavulanic-acid (AMC; 30 µg), Cephalexin (CL; 30 µg), Sulfamethoxazole-trimethoprim (SXT; 25 µg), Erythromycin (E; 15 µg), Colistin Sulfate (10 µg), Chloramphenicol (C; 30 µg), Cefotaxime (CTX; 5 µg), Ceftriaxone (CTR; 30 µg), Ciprofloxacin (CIP; 5 µg), Nalidixic acid (NA; 30 µg), Tetracycline (TE; 30 µg) (Liofilchem, France).

## Results

### Prevalence and Serotypes Distribution

*Salmonella* spp. was isolated from 11.83% (29/245) from egg samples and 12.24% (12/98) from over shoes. Out of the positive samples a total of 63 *Salmonella* isolates were obtained from eggshell (31 isolates), from yolk and albumen (13 isolates) and from over shoes (19 isolates) and then serotyped (Tables 1, 2).

Among the 63 strains isolated, 53 of them could be serotyped and they were found to belong to 8 serotypes namely *S.* Derby (36.51%), *S.* Typhimurium (11.11%), *S*. Brancaster (6.35%), *S.* Hato (6.35%), *S*. Kentucky (1.59%), *S*. Ouakam (1.59%), *S.* Cannstatt (1.59%), *S*. Essen (3.17%), and and serogroups such as F (12.70%) and O (3.17%). Ten strains were untypable (Table 3).

**Susceptibility to antimicrobial**

Of the total 53 *Salmonella* isolates subjected to antimicrobial susceptibility (Tables 4, 5), 41 (77.3%) *Salmonella* isolates were found to be resistant at least three antimicrobials. The antibiotics susceptibility results in this study highlighted the higher resistance of the avian *Salmonella* isolates from egg and laying hens isolates to Erythromycin (100 %), followed by Amoxicillin-clavulanic acid (52.6-59.1%), Ticarcillin (42.1-56.8%) and Tetracycline (42.1-45.4%). Resistance to multiple antimicrobial agents was predominantly seen in Derby, Kentucky and Typhimurium serotypes (Table 6).

**Discussion**

Human salmonellosis has been consistently associated with the consumption of poultry products worldwide (Zhao *et al.*, 2006; Im *et al.*, 2015). In the present study, *Salmonella* was detected in 11.8% of the eggs samples analysed and 12.24% in faecal matter from laying hens.

*Salmonella* isolates from both samples shown a high resistance to several antimicrobial. Previous study carried out by Kagambega *et al*., (2013) indicated resistance among *Salmonella* avian strains. Our study conform the persistence and increasing of resistance of *Salmonella* from poultry. Indeed, several authors observed this phenomenon from poultry and eggs. In India Bajaj *et al.*, (2003) reported strong resistance of *Salmonella* isolated in eggs to erythromycin (81.8%). Cardoso *et al.*, (2006); Akter *et al.*, (2007); Yoke-Kquen *et al.*, 2007; Singh *et al.*, (2010); Yildirim *et al.*, (2011); Adesiyun *et al.*, (2014); and Al *et al.*, (2016) also reported a high prevalence of erythromycin of 63.7-100%. Yildirim *et al.*, (2011) reported a high prevalence of tetracycline 67.6% and streptomycin 61.7%. It is, however, pertinent to mention that erythromycin is not routinely used in clinical settings and animal husbandry to prevent or treat salmonellosis but was used in the current study to characterize the isolates.

*Salmonella* Typhimurium is known to be able to cause high rates of mortality in early ages of broiler chickens (Padron, 1990). *Salmonella* Typhimurium was also reported with a prevalence of 7/63 (11.1%), higher than that reported by El-Sharkawy *et al.*, (2017) 58/615 (9.3%) in Egypt. *Salmonella* Derby is the predominant serotypes in egg samples (Long *et al.*, 2017). Derby was one of the main serotypes in the present study 23/63 (36.51%). *Salmonella* isolates were recovered not only from eggshells, but also from egg content. Previous studies revealed that under normal conditions of storage and moisture, *Salmonella* contaminating eggshells could migrate to the egg content (Im *et al.*, 2015). The environment of the layer farm was considered as a reservoir for *Salmonella* and could contribute to the horizontal/vertical dissemination, of *Salmonella* (Suresh *et al.*, 2011; Singh *et al.*, 2013), since *Salmonella* had the ability to persist in both host and non-host environments for its enhanced survival capabilities (Condell *et al.*, 2012). Furthermore, direct contact between egg belt and egg nest eggs were considered to be efficient mechanisms for the transmission of *Salmonella* (McWhorter *et al.*, 2015; Davies and Breslin, 2003). The prevalence of *Salmonella* in this study was however lower than 24.17%. Low prevalence was reported in Nigeria (Ekundayo and Ezeake, 2011), 0.3% from poultry eggs in Dhaka (Begum *et al.*, 2010), 7.7% recorded in South India (Suresh *et al.*, 2006), 3.84% and 5.5% among the chicken eggs from poultry farm and marketing in North India (Singh *et al.*, 2010).

The variation in the prevalence of *Salmonella* in eggs may be due to lack of awareness of the status of *Salmonella* in chicken eggs and the unhygienic situation in the farm. In contrast to modern farm egg collecting system, in our study it was observed eggs on farm soil smear by faecal matter. In addition eggs can stay in farms for several hours before their collect by farmers. The eggs-laying and management and storage practices in farm were signalled as factor of their contamination by faecal matter (Humphrey *et al.*, 1989; Al *et al.*, 2016; Tessema *et al.*, 2017).

The prevalence of resistant samples to tetracycline and streptomycin can be explained by their frequent administration in veterinary medicine (De Oliveira *et al*., 2010). This finding confirmed that in poultry, these drugs are used either for disease treatment or as growth promoters without prescription because they are cheap and easily affordable (Bouda *et al.*, 2019). This uncontrolled use of antibiotics in poultry farms leads to an increase of multidrug resistance, causing a negative impact on food products of animal origin. Resistance rates to nalidixic acid, Ceftriaxon, chloramphenicol, gentamycin, ciprofloxacin Sulfamethoxazole- trimethoprim of the isolates in the present study were low.

**Conclusion**

Based on the results of this study, it can be concluded that eggs and laying hens are carriers of antibiotic-resistant *Salmonella*. The level of contamination of egg with *Salmonella* species in this study calls for urgent need to control the level of *Salmonella* contamination of poultry farms in the study area. The high level resistance of the isolates to commonly used antibiotics is really alarming and has great public health significance if these microorganisms are transmitted to humans through food chain.

**Ethical Considerations**

Permission to conduct this study was obtained from the poultry farmers and the sellers of eggs; the study protocol was approved by the Ethical Committee of Burkina Faso.

**Competing Interests**

The authors declare that they have no competing interests.

**Authors' Contributions**

CB carried out strain isolation, characterization and drafted the manuscript, AK, NB and MC supervised and participated in writing the manuscript. All authors read, Commented on and approved of the final manuscript.

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Table 1 : Prevalence of *Salmonella* in chicken eggs from poultry farm.

| City (sample number) | Egg shells outer (%) | Albumen and Yolk (%) |
|---|---|---|
| Ouagadougou (n= 89) | 6 (6.74) | 0 (0.00) |
| Bobo Dioulassso (n=156) | 17 (10.90) | 6 (3.85) |
| Total (n= 245) | 23 (9.40) | 6 (2.45) |

Table 2 : Contaminated farms determinated by detection of *Salmonella* in chicken (laying hens) defecation at shed surfaces

| Samples sources | City, (number of farms visited) | Farms contaminated (%) |
|---|---|---|
| Poultry farms | Ouagadougou (n=28) | 6 (21.43) |
| | Bobo Dioulasso (n=70) | 6 (8.57) |
| Total | n=98 | 12 (12.24) |

Table 3: Details of different serotypes of *Salmonella* enterica obtained from eggs and laying hens.

| ***Salmonella* serovars** | Origin | | | |
|---|---|---|---|---|
| | Eggs | | Faeces | Total (%) |
| | Shell outer | Albumen and yolk | | |
| S. Brancaster | - | 2 | 2 | 4 (6.35) |
| S. Cannstatt | 1 | - | - | 1 (1.59) |
| S. Derby | 9 | 5 | 9 | 23 (36.51) |
| S. Hato | - | 1 | 3 | 4 (6.35) |
| S. Essen | 2 | - | - | 2 (3.17) |
| S. Kentucky | - | 1 | - | 1 (1.59) |
| S. Ouakam | 1 | - | - | 1(1.59) |
| S. Tyhimurium | 5 | 2 | - | 7 (11.11) |
| S Group F | 6 | 2 | - | 8 (12.7) |
| S Group O | 1 | - | 1 | 2 (3.17) |
| Untypable | 6 | - | 4 | 10 (15.87) |
| Total | 31 | 13 | 19 | 63 (100) |

Table 4 : Antibiogram results of *Salmonella* isolates from egg

| Antibiotics | Resistant | Intermediate | Sensitive |
|---|---|---|---|
| AZT (30µg) | 10 (27,7%) | 19 (43,2%) | 15 (34,1%) |
| AMC (30 µg) | 26 (59,1%) | - | 18 (40,9%) |
| TC (75µg) | 25 (56,8%) | - | 19 (43,2%) |
| IPM (10µg) | - | 7 (15,9%) | 37 (84,1%) |
| CL (30µg) | - | - | 44 (100%) |
| CTR (30µg) | 1 (2,3%) | 13 (29,5%) | 30 (68,2%) |
| CTX (5µg) | 15 (34,1%) | 11 (25%) | 18 (40,9%) |
| STR (10µg) | 4 (9,1%) | 26 (59,1%) | 14 (31,8%) |
| GEN (10µg) | 1 (2,3%) | 11 (25%) | 32 (72,7%) |
| C (30µg) | 2 (4,5%) | - | 42 (95,5%) |
| TE (30µg) | 20 (45,4%) | 5 (11,4%) | 19 (43,2%) |
| Na (30µg) | 1 (2,3%) | 1 (2,3%) | 42 (95,4%) |
| CIP (5µg) | 7 (15,9%) | 8 (18,2%) | 29 (65,9%) |
| E (15µg) | 44 (100%) | - | |
| SXT (25µg) | 4 (9,1%) | - | 40 (90,9%) |
| CS (10µg) | 8 (18,2%) | - | 36 (81,8%) |

Gentamicin: GEN, Streptomycin: STR, Aztreonam: AZT, Ticarcillin: TC, Imipenem: IPM, Amoxicillin–clavulanic-acid: AMC, Cephalexin: CL,,Sulfamethoxazole–trimethoprim: SXT, Colistin Sulfat: Cs, Chloramphenicol: C, Cefotaxim: CTX, Ceftriaxon: CTR, Ciprofloxacin: CIP, Nalidixic acid: NA, Tetracycline: TE

Table 5 : Antibiogram results of *Salmonella* isolates from fresh faeces of laying hens

| Antibiotics | Resistant | Intermediate | Sensitive |
|---|---|---|---|
| AZT (30µg) | 1 (5,3%) | 7 (36,8%) | 11 (57,9%) |
| AMC (30 µg) | 10 (52,6%) | - | 9 (47,%) |
| TC (75µg) | 8 (42,1%) | - | 11 (57,9%) |
| IPM (10µg) | - | 4 (21,1%) | 15 (78,9%) |
| CL (30µg) | - | - | 19 (100%) |
| CTR (30µg) | 1 (5,3%) | 2 (10,5%) | 16 (84,2%) |
| CTX (5µg) | 7 (36,8%) | 6 (31,6%) | 6 (31,6%) |
| STR (10µg) | - | 9 (47,4%) | 10 (52,6%) |
| GEN (10µg) | - | 3 (15,8%) | 16 (84,2%) |
| C (30µg) | - | - | 19 (100%) |
| TE (30µg) | 8 (42,1%) | 4 (21,1%) | 7 (36,8%) |
| Na (30µg) | - | - | 19 (100%) |
| CIP (5µg) | 1 (5,3%) | 3 (15,8%) | 15 (78,9%) |
| E (15µg) | 19 (100%) | - | - |
| SXT (25µg) | 2 (10,5%) | - | 17 (89,5%) |
| CS (10µg) | 3 (15,8%) | - | 16 (84,2%) |

Gentamicin: GEN, Streptomycin: STR, Aztreonam: AZT, Ticarcillin: TC, Imipenem: IPM, Amoxicillin–clavulanic-acid: AMC, Cephalexin: CL,,Sulfamethoxazole–trimethoprim: SXT, Colistin Sulfat: Cs, Chloramphenicol: C, Cefotaxim: CTX, Ceftriaxon: CTR, Ciprofloxacin: CIP, Nalidixic acid: NA, Tetracycline: TE

Tableau 6: Multiple antimicrobial resistance patterns of *Salmonella* serovars.

| Antimicrobial resistance pattern* | Number of resistant *Salmonella* serovars | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Brancaster (4) | Cannstatt (1) | Derby (20) | Essen (1) | Hato (4) | Kentucky (1) | Ouakam (1) | Tyhimurium (4) | Group F (8) | Group O (2) | Untypable (10) | Total (57) |
| E-AUG | | | 1 | | | | | | 1 | | 3 | |
| E- TC | | | | | 1 | | | 1 | 1 | | 1 | |
| E- TE | | | 4 | | 1 | | | | | 1 | | |
| E- AZT | | | | | | | | 1 | | | | |
| E- AUG- TC | | | 1 | | | | 1 | 1 | | | 1 | |
| E-AUG- TE | | | 1 | | | | | | | | | |
| E- AUG- CTX | | | 1 | | | | | | | | | |
| E- AUG- CS | | | 1 | | | | | | | | | |
| E- TC- CTX | | | 1 | | | | | | | | | |
| E- TC- TE | | | 2 | | | | | | | | | |
| E-TE-SXT | 2 | | | | | | | | | | | |
| E-CTX-TE | | | | 1 | | | | | | | | |
| E- AUG- TC- CTX | | 1 | | | 1 | | | | | | | |
| E- AUG- TC- TE | | | 2 | | | | | | | | | |
| E- AUG- TC- CIP | | | | | | | | | 1 | | | |
| E- AUG- TC- CS | | | | | | | | | 1 | | | |
| E- AUG- CTX- TE | | | 1 | | | | | | | | | |
| E- AUG- CTX- CS | | | | | | | | | | | 3 | |
| E- TC-TE- CS | | | | | 1 | | | | | | | |
| E- S- TE- SXT | 2 | | | | | | | | | | | |
| E- AZT- AUG- TC- CTX | | | 1 | | | | | | | | | |
| E- AUG- TC- CTX- CIP | | | | | | | | | | | 1 | |
| E- AZT- AUG- TC- CTX | | | | | | | | | | | 1 | |
| E- AUG- TC- CTX- TE | | | 2 | | | | | | | | | |
| E- TC- S- TE- SXT | | | | | | 1 | | | 1 | | | |
| E- TC- S- TE- SXT | | | | | | | | | | | | |
| E- AZT- AUG- TC- CTX- TE | | | 1 | | | | | | | | | |
| E- AZT-AUG- CRO- CTX- TE | | | 1 | | | | | | | | | |
| E- AUG- AZT- TC- CRO- CTX- CIP- CS | | | 1 | | | | | | | | | |
| E- AUG- AZT- TC- CTX- CIP- CS | | | | | | | | | 1 | | | |
| E- AUG- TC- CTX- TE-CIP- CS | | | | | | | | | 1 | | | |
| E- AZT-AUG- TC- CTX-TE- CIP | | | | | | | | | | 1 | | |
| E- AUG- TC- CTR- GEN- TE- CIP | | | | | | | | 1 | | | | |
| E- AUG- AZT-TC- CTX- S- CIP- CS | | | | | | | | | 1 | | | |
| Resistance to 3 - 4 antibiotics | 4 | 1 | 10 | 1 | 2 | | 1 | 1 | 2 | - | 4 | 26 |
| Resistance to 5 or more antibiotics | - | - | 6 | | | 1 | | 1 | 3 | 1 | 2 | 15 |