

# Antimicrobial resistance pattern of *Salmonella* spp. isolated from poultry farms in Abakaliki, Nigeria

EMMANUEL NNABUIKE UGBO<sup>1</sup>, MUSTOFA HELMI EFFENDI<sup>2</sup>, ADIANA MUTAMSARI WITANINGRUM<sup>2</sup>,  
WIWIEK TYASNINGSIH<sup>3,\*</sup>, BERNARD NNABUIFE AGUMAH<sup>1</sup>, AGATHA IFUNANYA UGBO<sup>4</sup>,  
CHARITY CHINYERE NNABUGWU<sup>5</sup>, DIVINEGIFT OLUCHI OKATA-NWALÍ<sup>5</sup>

<sup>1</sup>Department of Applied Microbiology, Faculty of Science, Ebonyi State University. P. M. B. 053, Abakaliki 481101, Ebonyi, Nigeria

<sup>2</sup>Department of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Mulyorejo, Kampus C FKH-UNAIR, Surabaya 60115, East Java, Indonesia

<sup>3</sup>Department of Veterinary Microbiology, Faculty of Veterinary Medicine, Universitas Airlangga. Jl. Mulyorejo, Kampus C FKH-UNAIR, Surabaya 60115, East Java, Indonesia. Tel.: +62-315-992785, Fax.: +62-315-993015, \*email: witya\_kh@yahoo.com

<sup>4</sup>Department of Microbiology and Parasitology, David Umahi Federal University of Health Sciences Uburu. Ebonyi, Nigeria

<sup>5</sup>Department of Microbiology, Faculty of Bioscience, Alex Ekwueme Federal University Ndufu Alike. Ebonyi, Nigeria

Manuscript received: 20 August 2023. Revision accepted: 30 September 2023.

**Abstract.** Ugbo EN, Effendi MH, Witaningrum AM, Tyasningsih W, Agumah BN, Ugbo AI, Nnabugwu CC, Okata-Nwali DO. 2023. Antimicrobial resistance pattern of *Salmonella* spp. isolated from poultry farms in Abakaliki, Nigeria. *Biodiversitas* 24: 5207-5214. *Salmonella* is a zoonotic foodborne pathogen that can cause serious illness in humans and animals worldwide. Antimicrobial resistance caused by microorganisms has greatly challenged veterinary and human medicine regarding disease treatments. The objective of this study was to evaluate the antimicrobial resistance pattern of *Salmonella* spp. isolated from poultry farms in Abakaliki, Nigeria. One hundred eighty samples (90 broiler feces; 90 broiler cloacal swabs) were collected from three (3) poultry farms in the Abakaliki metropolis. Samples were analyzed and identified for the presence of *Salmonella* isolates using standard microbiological and biochemical analysis. The antimicrobial sensitivity test of *Salmonella* isolates against the selected antibiotics was done using the Kirby-Bauer disk diffusion method on Mueller Hinton agar. The results showed 61 samples (33.9 %) were positive for *Salmonella*. Feces and cloacal swab samples positive for *Salmonella* were 46.7% and 21.1%, respectively. Multidrug resistance *Salmonella* (MDR) strains had an overall prevalence of samples (16.2%), i.e., 7 samples of broiler feces had 7 (16.7%) and 3 samples of cloacal swab (15.8%). Thus, all the MDR *Salmonella* isolates were 100% resistant to tetracycline and ampicillin. The multiple antibiotic-resistant indexes of the MDR were 0.51. This study indicated that broilers chicken and their products were potential sources of human salmonellosis and sources for transmitting MDR *Salmonella* in Abakaliki, Nigeria. Poultry farmers should use antibiotics in appropriate dosages in their poultry farms.

**Keywords:** Antimicrobial resistance, human illness, MDR, poultry farms, *Salmonella*

## INTRODUCTION

*Salmonella* species cause two major diseases: gastroenteritis, non-typhoid Salmonellosis or *Salmonella* poisoning, and human typhoid fever (Yanestria et al. 2019; Wibisono et al. 2023). Many people become infected by *Salmonella* mainly by consuming contaminated water, fruits/vegetables, or foods like seafood, undercooked beef, poultry, and raw eggs (Robertson et al. 2018). Infections caused by these bacteria or their toxins are known as Salmonellosis. An important characteristic of *Salmonella* is its ability to grow and multiply outside its host, thus having higher survival chances (Hussein et al. 2009).

Poultry products are a leading source of protein in Nigeria, as in many developing countries, because they are cheap and readily available (Gymoese et al. 2017). Chicken meats, such as broiler chickens and laying hens, are included as one of the livestock products that are widely consumed. However, it is a primary medium for the spread of the foodborne disease caused by *Salmonella* infection (Salmonellosis) through the food production chain (Sin et al. 2020; Wibisono et al. 2021). In Nigeria, *Salmonella*

infections can be transmitted through contaminated poultry feeds, dust, the presence of carriers (rodents and insects), water, contact with the infected birds/feces, animal-to-human contact, from poor or unclean poultry farms by the poultry workers that can quickly spread to the environment either by human contact or consumption of contaminated poultry meat (Jibril et al. 2020). Cross-contamination of poultry products can occur during handling, transportation, and slaughter processes through equipment such as contaminated tables, cutting materials, and packaging (Fagbamila et al. 2017). Studies have estimated that *Salmonella* organisms cause 93.8 million human infections and 155,000 to 230,000 deaths annually (Jibril et al. 2020; Fanissa et al. 2022).

The primary source of these infections was improperly sanitized grade A table eggs served raw or lightly cooked. Many studies have proven that *Salmonella* species contaminate eggs from the infected reproductive tissue of hens, rather than the shell, to the contents of contaminated eggs (Idowu et al. 2017). The widespread use of antibiotics in human and veterinary medicine increased resistant *Salmonella* strains isolated from human and environmental

sources. Poultry and other animals fed with feed containing antibiotics often had multiple resistances to antibiotics (Wibisono et al. 2020a; Permatasari et al. 2020; Riwu et al. 2020; Ansharieta et al. 2021). Most cases of non-typhoidal salmonellosis are associated with outbreaks from contaminated meat, animal products, and cross-contamination from food contaminated with *Salmonella* (Wibisono et al. 2023). In developing countries such as Africa and southern Asia, salmonellosis infection threatens public health, with an estimated 33 million cases annually (Tack et al. 2020). In Nigeria, the morbidity associated with *Salmonella* disease continues to increase, leading to death. As in other countries, first-line antibiotics, i.e., chloramphenicol, co-trimoxazole, and third-generation cephalosporins, are used to treat patients. However, the efficacy of some of these drugs is still not showing promising results following the emergence of multidrug resistance of *Salmonella* strains. In treating severe *Salmonella* infections, fluoroquinolones are efficacious both in vitro and in vivo, although strains with reduced susceptibility to ciprofloxacin have been reported in several countries (Usha et al. 2008). Research on foodborne illness related to *Salmonella* infection caused by contact with poultry feces or consumption of poultry meat or its products is still limited in Abakaliki, Ebonyi State, Nigeria. Therefore, there is a need to evaluate the antimicrobial resistance pattern of *Salmonella* species isolated from poultry farms in Abakaliki, Nigeria. The results of this study provided the level of multidrug-resistant *Salmonella* in poultry farms in Nigeria, suggesting best practices to mitigate the use of antibiotics in dealing with outbreaks of *Salmonella* infections in poultry farms.

## MATERIALS AND METHODS

### Ethical approval

Feces and cloacal swabs of broiler chickens were used in this study; hence, ethical approval was not necessary. The samples were collected from three poultry farms in Abakaliki, Nigeria.

### Study sites

The study was conducted at Abakaliki, the Ebonyi State capital in Southeastern Nigeria. Its geographical coordinates are a longitude of 8°06'E and a latitude of 6°20'N with a tropical climate, i.e., rainy season (April to October) and dry season (November to March). Its vegetation is the sub-savannah rainforest. The state's estimated population was 2.2 million in the 2006 census (National Bureau of Statistics 2012).

**Study design:** A cross-sectional study was conducted to isolate *Salmonella* from poultry farms between August and November 2022. Chicken feces and cloacal swabs were collected randomly from three (3) poultry farms, i.e., Nkaliki, Ishieke, and Kpirikpiri, within the Abakaliki metropolis.

**Sample collection:** Samples were randomly collected from the deep litter that is not properly cleaned, which is located in open spaces. Chickens were fed with

mash/grains. One hundred and eighty samples (90 broiler chicken feces and 90 broiler cloacal swabs) were collected from three (3) different poultry farms in the Abakaliki metropolis, which includes Nkaliki - 60 samples (30 chicken feces; 30 cloacal swabs), Ishieke - 60 samples (30 chicken feces; 30 cloacal swabs), and Kpirikpiri - 60 samples (30 chicken feces; 30 cloacal swabs). Sampling was carried out using a sterile swab, properly labeled. Samples were transported within one hour of collection to the Department of Applied Microbiology Laboratory using a thermo-box at a temperature of 4°C for bacteriological analysis. The samples were studied for the presence of *Salmonella* using cultural-based methods, Gram staining, and biochemical identification processes.

### Isolation and identification of *Salmonella* isolates

The collected broiler chicken feces and cloacal swab samples were inoculated into 5 mL of selenite F broth (Oxoid, UK) for pre-enrichment and incubated overnight. A loopful of the overnight grown organisms were further inoculated, sub-cultured onto *Salmonella-Shigella* agar (Sigma Aldrich, German), and incubated aerobically for 18 to 24 h at 37°C. The suspected colonies of *Salmonella* species on the *Salmonella-Shigella* agar were non-lactose fermenters with a blackish appearance on the media. The colonies of *Salmonella* species were further analyzed for Gram staining, morphological, and biochemical characteristics by subjecting them to H<sub>2</sub>S production, citrate, urease, indole, motility test, and sugar fermentation tests (Fanissa et al. 2022). The isolates that showed citrate positive, H<sub>2</sub>S production, and motility but indole negative reaction were considered presumptive *Salmonella* species. They were further sub-cultured onto Nutrient agar (Oxoid, UK) and purified for further studies.

### Antimicrobial susceptibility testing

Antimicrobial susceptibility assay was conducted using the following antibiotics: ampicillin (AMP-30 µg); ciprofloxacin (CIP-5 µg); gentamicin (CN-15 µg); imipenem (IPM-10 µg); ofloxacin (OFX-5 µg); nitrofurantoin (FT-100 µg), sulphamethoxazole-trimethoprim (SXT-25 µg); tetracycline (TE-30 µg); chloramphenicol (C-30 µg); cefotaxime (CTX-30 µg) (Oxoid, UK). The antibiotic sensitivity of the *Salmonella* isolates was determined using the Kirby-Bauer disc diffusion method. The Mueller Hinton Agar (MHA) (Oxoid, UK) was prepared according to the manufacturer's standard. The 0.5 McFarland standardized equivalent of the *Salmonella* isolates was seeded onto MHA using a sterile swab stick. Ten (10) different antibiotic paper disks were carefully placed onto MHA prepared in 90 mm Petri dishes. The antibiotic disks were placed at a distance of 30 mm away from each other and 15 mm measured from the edge of the plate. The plates with the antibiotic disk were allowed to stay 10 minutes before it was inverted and incubated at 37°C for 18 to 24 hours. After incubation, the zone of inhibition was determined. The caliper was used to measure the diameter of zone inhibition and was interpreted as susceptible or resistant using CLSI guidelines (CLSI 2018).

### Determination of multiple antibiotics resistance index

The multiple antibiotic resistance index (MARI) was calculated to determine the antibiotic resistance of the isolated multidrug resistance *Salmonella* species. The formula of MARI is  $a/b$ , where  $a$  represents the number of antibiotics to which the *Salmonella* isolates are resistant; 'b' represents the total number of antibiotics used to evaluate bacteria (Ejikegwu et al. 2018).

## RESULTS AND DISCUSSION

This research identified *Salmonella* species isolates using morphological characters on *Salmonella-Shigella* agar (black-centered coloration pigments). The *Salmonella* isolates were gram-negative rod-shaped. Biochemical properties of *Salmonella* include citrate positive, H<sub>2</sub>S production positive, urease negative, Voges Proskauer negative, indole negative reaction, and motility positive. The morphological and biochemical analysis results showed 61 samples (33.9%) were identified as *Salmonella*. The number of samples positive for *Salmonella* from the Nkaliki-A farm, Ishieke-B, and Kpirikpiri-C were 20 samples (33.3%), 26 samples (43.3%), and 15 samples (25.0%), respectively (Table 1). Broiler feces harbored more *Salmonella* isolate at 46.7% than cloacal swabs (21.1%). Multidrug resistance (MDR) *Salmonella* isolates were also obtained in the three farms studied, with a prevalence of 16.4%. Based on the location of the farms, multidrug resistance (MDR) *Salmonella* isolates in the Nkaliki-A farm was 4 (20.0%), whereas Ishieke-B was 4 (15.4%), and Kpirikpiri-C reported 2 (13.3%). The MDR *Salmonella* isolates from poultry feces and cloacal swabs were 7 (16.7%) and 3 (15.8%), respectively (Table 2). Antimicrobial susceptibility testing on the organisms showed that the isolates were 75% to 100% susceptible to ciprofloxacin, imipenem, and chloramphenicol and 100% susceptible to gentamicin. However, the isolates were 50% to 100% resistant to only ofloxacin and cefotaxime, 25% to

75% resistant to nitrofurantoin, sulphamethoxazole-trimethoprim, and 100% resistant to ampicillin, tetracycline (Table 3). The resistant patterns of *Salmonella* isolates obtained in this study include AMP-FT-OFX-SXT-TE; TE-AMP-SXT-FT; AMP-IPM-FT-SXT-TE; AMP-OFX-CTX-FT-SXT-TE; AMP-SXT-CTX-TE; AMP-SXT-TE-CTX-FT; AMP-FT-SXT-TE-C; AMP-CIP-IPM-OFX-TE-CTX; AMP-CTX-OFX-SXT-TE; AMP-FT-OFX-TE-CTX-C. The isolates exhibited multiple antibiotic resistance indexes (MARI) ranging from 0.40 to 0.60 with average MARI of 0.51 (Table 4). The growth appearance of *Salmonella* isolates on *Salmonella-Shigella* agar is represented in Figure 1, while the antimicrobial susceptibility appearance of *Salmonella* species isolated from poultry is presented in Figure 2.



**Figure 1.** The growth appearance of *Salmonella* isolates on *Salmonella-Shigella* agar (SSA) showing black colonies

**Table 1.** Prevalence of *Salmonella* species from poultry farms based on location in Abakaliki, Nigeria

Location	Farms	No of the samples analyzed	No positive for <i>Salmonella</i> species	Percentage prevalence (%)	Multidrug resistance <i>Salmonella</i> (%)
Nkaliki	A	60	20	33.3	4 (20.0)
Ishieke	B	60	26	43.3	4 (15.4)
Kpirikpiri	C	60	15	25.0	2 (13.3)
Total	3	180	61	33.9	10 (16.2)

**Table 2.** Prevalence of *Salmonella* species from poultry farms based on the sample sources

Sample source	Number of samples analyzed	Number positive for <i>Salmonella</i> species	Percentage prevalence of <i>Salmonella</i> spp (%)	Multidrug resistance <i>Salmonella</i> (%)
Droplet swab	90	42	46.7	7 (16.7)
Cloacal swab	90	19	21.1	3 (15.8)
Total	180	61	33.9	10 (16.2)

**Table 3.** Antibiotic susceptibility of multidrug resistance *Salmonella* species isolated from various poultry farms in Abakaliki, Nigeria

Location/ Farm	C		CIP		SXT		IMP		OFX		FT		AMP		CN		CTX		TE	
	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
Nkaliki =A	3(75)	1(25)	3(75)	1(25)	1(25)	3(75)	3(75)	1(25)	2(50)	2(50)	2(50)	2(50)	0(0)	4(100)	4(100)	0(0)	2(50)	2(50)	0(0)	4(100)
Ishieke =B	3(75)	1(25)	4(100)	0	1(25)	3(75)	3(75)	1(25)	2(50)	2(50)	1(25)	3(75)	2(0)	4(100)	4(100)	0(0)	2(50)	2(50)	0(0)	4(100)
Kpirikpiri =C	2(100)	0(0)	2(100)	0(0)	1(25)	3(75)	2(100)	0(0)	2(100)	0(0)	1(50)	1(50)	0(0)	3(100)	2(100)	0(0)	0(0)	2(100)	0(0)	2(100)

Note: Susceptible (S); Resistant (R); Ampicillin (AMP); Ciprofloxacin (CIP); Gentamicin (CN); Imipenem (IPM); Ofloxacin (OFX); Nitrofurantoin (FT), Sulphamethoxazole-Trimethoprim (SXT); Tetracycline (TE); Chloramphenicol (C); Cefotaxime (CTX); Farm A = Nkaliki; Farm B = Ishieke; Farm C = Kpirikpiri

**Table 1.** Multiple antibiotic resistance index/patterns of multidrug-resistant *Salmonella* species isolated from poultry farms

Code	Isolates	Number of antibiotics that isolates were resistant to (a)	Total number of antibiotics tested (b)	MAR index (a/b)
FAD 1	<i>Salmonella</i>	AMP-FT-OFX-SXT-TE	10	0.50
FBD 2	<i>Salmonella</i>	TE-AMP-SXT-FT	10	0.40
FBC 3	<i>Salmonella</i>	AMP-IPM-FT-SXT-TE	10	0.50
FAD 4	<i>Salmonella</i>	AMP-OFX-CTX-FT-SXT-TE	10	0.60
FCC 5	<i>Salmonella</i>	AMP-SXT-CTX-TE	10	0.40
FCD 6	<i>Salmonella</i>	AMP-SXT-TE-CTX-FT	10	0.50
FAD 7	<i>Salmonella</i>	AMP-FT-SXT-TE-C	10	0.50
FAC 8	<i>Salmonella</i>	AMP-CIP-IPM-OFX-TE-CTX	10	0.60
FBD 9	<i>Salmonella</i>	AMP-CTX-OFX-SXT-TE	10	0.50
FBD10	<i>Salmonella</i>	AMP-FT-OFX-TE-CTX-C	10	0.60
The total prevalence of multidrug resistance <i>Salmonella</i> spp. (10/61) = 16.2%				Average: 0.51

Note: FAD-Farm A Droplet; FBD-Farm B Droplet; FCD-Farm C Droplet; FAC-Farm A Cloacal; FBC-Farm B Cloacal; FCC-Farm C Cloacal



**Figure 2.** Antimicrobial susceptibility of *Salmonella* species isolated from poultry. CN - Susceptible; AMP, STX, FX, CTX - Resistance

## Discussion

*Salmonella* infections remain a major global public health problem, and poultry is the primary transmission source to humans. This study showed that the overall prevalence of *Salmonella* species from the three poultry farms in Abakaliki was relatively high, with a percentage prevalence of 33.9. A previous study showed that *Salmonella* species prevalence in poultry farms in other parts of Nigeria was 43.6% (Fagbamila et al. 2017). Another study by Useh et al. (2016) reported a high prevalence of *Salmonella* species from poultry sources in Nigeria. Asogwa et al. (2022) reported 45% *Salmonella* species prevalence in Enugu, Nigeria. A study by Sohail et al. (2021) reported the overall prevalence of *Salmonella* in poultry was 25.82%. A study conducted at Sokoto State in Nigeria reported *Salmonella* contamination to be 28.8% (Faleke et al. 2017). A high prevalence of *Salmonella* species from poultry farms has also been reported in other sub-Saharan African countries such as Ghana (44.0%)

(Odoch et al. 2017), Uganda (20.7%) (Egualé 2018) and Ethiopia (14.6%) (Andoh et al. 2016). Studies from developing Asian countries reported a prevalence of 46.3% in central Vietnam (Barua et al. 2012) and 42% and 18% in Bangladesh, respectively (Lettini et al. 2016; Sarker et al. 2021). Fanissa et al. (2022) reported a low prevalence of *Salmonella* species in Surabaya, Indonesia, with a frequency of 11.3%. Other researchers have observed a similar prevalence of *Salmonella* species; poultry samples 23.53% (Al-Mamun et al. 2017); poultry production 37.9% (Mahmud et al. 2011), chickens 11.54% (Abd El-Aziz et al. 2021) and broiler farms 31.25% (Mridha et al. 2020). Prevalence of *Salmonella* bacteria was detected in 21.3% of chickens analyzed (Telli et al. 2022). The variation in the prevalence of *Salmonella* species, as observed in this study, is a result of the sites of the samples and the area/location of the farms. Thus, the altitude, practice, and level of hygiene practice by the owners of poultry farms and workers are also the major factors contributing to *Salmonella* prevalence.

Furthermore, these studies also reported the *Salmonella* species prevalence based on the sample collection or source site. We observed that the broiler droplet harbored more *Salmonella* species, with a prevalence of 46.7%, while the cloacal swab harbored 21.1%. Slader et al. (2002) reported a 56% prevalence of *Salmonella* species from chicken feces. A similar prevalence of *Salmonella* species has been obtained in chicken cloacal swabs at 26.6% (Akond et al. 2012). Ibrahim et al. (2021) detected *Salmonella* bacteria from chicken cloacal with a prevalence of 6.5%. Poultry farm environment and contact surfaces were also suspected as the potential for possible cross-contamination. However, the high prevalence of *Salmonella* in poultry feces over the cloacal swab, as observed in this study, suggested that frequent contamination of *Salmonella* occurs along the brooding and growing stage due to poor hygiene practices by farm workers.

It should be noted that the prevalence of *Salmonella* has increased rapidly over the years in Nigeria, suggesting that poultry products from markets or farms can be a source of transmission of *Salmonella* infections to humans. Wibisono et al. (2020b) stated that shedding *Salmonella* or other

enteric pathogens in live poultry increased under stress or poorly cared conditions during the growing period. Salmonellosis is a zoonotic disease caused by *Salmonella* species that can affect humans and animals. Through contact with the environment around animals or contaminated objects around poultry farms, indirect *Salmonella* transmission occurred (Abd El-Ghany 2020). Ahmed et al. (2019) reported that chickens and poultry farms/environments were important reservoirs of *Salmonella* in Nigeria. It is similar to *Salmonella* species from chicken feces and cloacal swabs, as observed in this study.

This study identified multidrug resistance *Salmonella* bacteria with an overall prevalence of 16.2%, with different prevalences from chicken feces and cloacal swabs of 16.7 and 15.8 %, respectively. A study by Pławinska-Czarnak et al. (2022) reported similar prevalences of *Salmonella*. One of the most critical health problems in the world is antimicrobial resistance due to *Salmonella* infection (EFSA and ECDDC 2022; Kong-Ngoen et al. 2022). Data from the European Union show that the emergence of multidrug resistance in *Salmonella* from pigs, cattle, and broiler chickens is mainly similar to the emergence of *Salmonella* resistance in various foodstuffs and humans (EFSA and ECDDC 2022). Multidrug-resistant *Salmonella* seriously threatens public health through foodborne infections (Lai et al. 2014). Multidrug-resistant *Salmonella* strains are increasingly isolated from poultry (Yang et al. 2019), beef (Barilli et al. 2018) and pork (Campos et al. 2019). Multiple-resistant *Salmonella* strains were often responsible for severe and even fatal human systematic infections (Effendi et al. 2019). The excessive or inappropriate use of antibiotics in human and veterinary medicine has contributed significantly to the emergence of multidrug-resistant bacteria. Studies have reported Salmonellosis outbreaks in humans after contact with live birds (Medalla et al. 2017; Putra et al. 2020). Multidrug resistance in *Salmonella* strains showed various resistant index patterns against the antibiotic classes tested, including beta-lactam, carbapenem, fluoroquinolones, tetracycline, aminoglycosides, sulfonamides, and nitrofurans. The multidrug resistance *Salmonella* strain isolated in this study showed 75% to 100% susceptibility to fluoroquinolones (ciprofloxacin), carbapenem (imipenem), aminoglycosides (gentamicin) in accordance with the report of Wibisono et al. 2021. A previous study by Abd El-Aziz et al. (2021) reported multidrug-resistant *Salmonella* in chicken (Abd El-Aziz et al. 2021).

The multidrug resistance *Salmonella* isolates in this research were mostly resistant to penicillins (ampicillin), tetracycline, fluoroquinolones (ofloxacin), sulfonamides (sulphamethoxazole/trimethoprim) and nitrofurans (nitrofurantoin) with their resistant ranging from 50% to 100%. The resistant patterns includes; AMP-FT-OFX-SXT-TE; TE-AMP-SXT-FT; AMP-IPM-FT-SXT-TE; AMP-OFX-CTX-FT-SXT-TE; AMP-SXT-CTX-TE; AMP-SXT-TE-CTX-FT; AMP-FT-SXT-TE-C; AMP-CIP-IPM-OFX-TE-CTX; AMP-CTX-OFX-SXT-TE; AMP-FT-OFX-TE-CTX-C. A study conducted in Surabaya, Indonesia, has reported a similar multidrug resistance pattern against

*Salmonella* strains isolated from poultry across three antimicrobial classes such as TE-CIP-SXT, TE-CHL-SXT, and four antimicrobial classes ATM-TE-CIP-CHL -SXT, TE-CIP-CHL-SXT (Fanissa et al. 2022). Another study reported the presence of multidrug resistance of the *Salmonella* strain with resistance patterns as AMP-SXT-CTX-C-TE; AMP-CTX-C-LEV-STX; C-TE-STX and AMP-C-TE (Nguyen et al. 2021). The high level of resistance found in the isolates against ampicillin and tetracycline was due to the frequent usage of antibiotics to treat infections in poultry farms. A similar resistant percentage has been reported in *Salmonella* isolated from poultry against tetracycline (60.33%), ampicillin (58.61%), and moderate resistance against gentamicin and chloramphenicol (Siddiky et al. 2022). It is in line with the results of this study. A study by Sohail et al. (2021) showed that *Salmonella* isolates had high levels of resistance against ciprofloxacin, ampicillin cefotaxime, ceftazidime, trimethoprim/sulfamethoxazole in poultry. Musawa et al. (2021) reported the presence of multidrug-resistant *Salmonella* strains in poultry highly resistant to penicillin and oxytetracycline. This study showed that the average multiple antibiotic resistance indexes (MARI) of multidrug-resistant *Salmonella* species isolated from the poultry farms were 0.51. The antimicrobial resistance of MDR *Salmonella* strain from poultry ranged from 0.13 to 0.63 (Abd El-Aziz et al. 2021) and is similar to the results of the present study. Antimicrobial-resistant *Salmonella* in poultry farms (feces and cloacal swabs) is a potential threat to poultry farmers, workers, and public health, especially immune-compromised who consume it as a meat and protein source. Thus, the government should maintain/monitor the rational use of antibiotics in poultry farms, proper meat handling/cooking practices, and provide regulatory agencies that could enforce the proper use of antimicrobials in livestock. A national surveillance network using one health approach should be implemented.

In conclusion, there is a high prevalence of *Salmonella* species in poultry farms in the Abakaliki metropolis with an overall frequency of 33.9%, with the occurrence of multidrug resistance *Salmonella* at 16.2%. MDR isolates were 100% resistant to ampicillin and tetracycline. Poultry farms, workers, and birds contribute to transmitting MDR *Salmonella* spp. to the community. The presence of MDR *Salmonella* species can lead to loss of income and threat to public health. Thus, we recommend that poultry farmers conduct proper hygiene practices and appropriate use of antibiotics, which is necessary in veterinary and human medicine to prolong the usefulness of antibiotics in treating infectious diseases.

## ACKNOWLEDGEMENTS

The authors would like to acknowledge Ebonyi State University, Abakaliki Nigeria, and Universitas Airlangga, Indonesia, for their support. This study was partly supported by the *Penelitian Hibah Mandat* funding from *Lembaga Penelitian dan Pengabdian Masyarakat*, Universitas Airlangga, Indonesia.

## REFERENCES

- Abd El-Aziz NK, Tartor YH, Gharieb RMA, Erfan AM, Khalifa E, Said MA, Ammar AM, Samir M. 2021. Extensive drug-resistant *Salmonella enterica* isolated from poultry and humans: Prevalence and molecular determinants behind the co-resistance to ciprofloxacin and tigecycline. *Front Microbiol* 12: 738784. DOI: 10.3389/fmicb.2021.738784.
- Abd El-Ghany WA. 2020. Salmonellosis: A foodborne zoonotic and public health disease in Egypt. *J Infect Dev Ctries* 14 (7): 674-678. DOI: 10.3855/jidc.12739.
- Ahmed AO, Raji MA, Mamman PH, Kwanashie CN, Raufu IA, Aremu A, Akorede GJ. 2019. Salmonellosis: Serotypes, prevalence and multidrug resistant profiles of *Salmonella enterica* in selected poultry farms, Kwara State, North Central Nigeria. *Onderstepoort J Vet Res* 86 (1): 1-8. DOI: 10.4102/ojvr.v86i1.1667.
- Akond MA, Shirin M, Alam S, Hassan SMR, Rahman MM, Hoq M. 2012. Frequency of drug resistant *Salmonella* spp. isolated from poultry samples in Bangladesh. *Stamford J Microbiol* 2 (1): 15-19. DOI: 10.3329/sjm.v2i1.15207.
- Al-Mamun MA, Kabir SML, Islam MM, Lubna M, Islam SS, Akhter AT, Hossain MM. 2017. Molecular identification and characterization of *Salmonella* species isolated from poultry value chains of Gazipur and Tangail districts of Bangladesh. *Afr J Microbiol Res* 11 (11): 474-481. DOI: 10.5897/AJMR2017-8431.
- Andoh LA, Dalsgaard A, Obiri-Danso K, Newman MJ, Barco L, Olsen JE. 2016. Prevalence and antimicrobial resistance of *Salmonella* serovars isolated from poultry in Ghana. *Epidemiol Infect* 144 (15): 3288-3299. DOI: 10.1017/S0950268816001126.
- Ansharieta R, Ramandinianto SC, Effendi MH, Plumeriastuti H. 2021. Molecular identification of bla CTX-M and bla TEM genes encoding extended-spectrum  $\beta$ -lactamase (ESBL) producing *Escherichia coli* isolated from raw cow's milk in East Java, Indonesia. *Biodiversitas* 22 (4): 1600-1605. DOI: 10.13057/biodiv/d220402.
- Asogwa NV, Udeani TK, Obeagu EI, Asogwa BE, Chukwueze CM. 2022. Prevalence of *Salmonella* species infection in poultry farming systems in Enugu metropolis Nigeria. *Eur J Pharm Med Res* 9 (4): 87-91.
- Barua H, Biswas PK, Olsen KEP, Christensen JP. 2012. Prevalence and characterization of motile *Salmonella* in commercial layer poultry farms in Bangladesh. *PLoS ONE* 7 (4): e0035914. DOI: 10.1371/journal.pone.0035914.
- Barilli E, Bacci C, Villa ZS, Meriardi G, D'Incau M, Brindani F, Vismarra A. 2018. Antimicrobial resistance, biofilm synthesis and virulence genes in *Salmonella* isolated from pigs bred on intensive farms. *Ital J Food Saf* 7: 131-137. DOI: 10.4081/ijfs.2018.7223.
- Campos J, Mourão J, Peixe L, Antunes P. 2019. Non-typhoidal *Salmonella* in the pig production chain: A comprehensive analysis of its impact on human health. *Pathogens* 8: 19. DOI: 10.3390/pathogens8010019.
- Clinical and Laboratory Standards Institute (CLSI). 2018. Performance standards for antimicrobial susceptibility testing 28th edition. Clinical and Laboratory Standards Institute.
- Effendi MH, Harijani N, Triningtya NP, Tyasningsih W, Plumeriastuti H. 2019. Prevalence of pathogenic *Escherichia coli* isolated from sub clinic mastitis in East Java Province, Indonesia. *Indian Vet J* 96 (3): 22-25. DOI: 10.13057/biodiv/d220137.
- Eguale T. 2018. Non-typhoidal *Salmonella* serovars in poultry farms in central Ethiopia: Prevalence and antimicrobial resistance. *BMC Vet Res* 14 (1): 1-8. DOI: 10.1186/s12917-017-1323-x.
- Ejikegwu C, Afukwa N, Nwuzo A, Ovia K, Adonu C, Ujam T, Nworie C, Agbai EN, Nwankwo C, Onwuenunor BO. 2018. Phenotypic detection of metallo- $\beta$ -lactamase (MBL) and AmpC enzymes among abattoir isolates of *Escherichia coli* and *Klebsiella* species in Abakaliki, Nigeria. *Trends Med* 18: 1-4. DOI: 10.15761/TiM.1000124.
- European Food Safety Authority (EFSA); European Centre for Disease Prevention and Control (ECDC) (2022). The European union summary report on antimicrobial resistance in zoonotic and indicator bacteria from humans, animals and food in 2018/2019. *EFSA J* 20. DOI: 10.2903/j.efsa.2022.7209.
- Fagbamila IO, Barco L, Mancin M. 2017. *Salmonella* serovars and their distribution in Nigerian commercial chicken layer farms. *PLoS One* 12 (3): e0173097. DOI: 10.1371/journal.pone.0173097.
- Faleke OO, Jolayemi KO, Igoh YO, Jibril AH, Ayedun JO. 2017. *Salmonella* species on meat contact surfaces and processing water in Sokoto main market and abattoir, Nigeria. *Macedonian Vet Rev* 40 (1): 59-65. DOI: 10.1515/macvetrev-2017-0011.
- Fanissa F, Effendi MH, Tyasningsih W, Ugbo EB. 2022. Multidrug-resistant *Salmonella* species from chicken meat sold at Surabaya Traditional Markets, Indonesia. *Biodiversitas* 23: 2823-2829. DOI: 10.13057/biodiv/d230606.
- Gymose P, Sorensen G, Litrup E, Olsen JE, Nielsen EM, Torpdahl M. 2017. Investigation of outbreaks of *Salmonella enterica* serovar typhimurium and its monophasic variants using whole-genome sequencing, Denmark. *Emerg Infect Dis* 23 (10): 1631-1639. DOI: 10.3201/eid2310.161248.
- Hussein M, Hala K, Khalil A. 2009. Characterization of *Salmonella* Lipopolysaccharide isolated from poultry farms in Jordan. *Dirasat Pure Sci* 36 (1): 30-37.
- Ibrahim S, Wei Hoong L, Lai Siong Y, Mustapha Z, Zalati CWS, Aklilu E, Mohamad M, Kamaruzzaman NF. 2021. Prevalence of Antimicrobial Resistance (AMR) *Salmonella* spp. and *Escherichia coli* isolated from broilers in the East Coast of Peninsular Malaysia. *Antibiotics* 10 (5): 579. DOI: 10.3390/antibiotics10050579.
- Idowu OF, Lisa B, Marzia M, Jacob K, Sati SN, Paola Z. 2017. *Salmonella* serovars and their distribution in Nigerian commercial chicken layer farms. *PLoS One* 12 (3): e0173097. DOI: 10.1371/journal.pone.0173097.
- Jibril AH, Okeke IN, Dalsgaard A, Kudirkiene E, Akinlabi OC. 2020. Prevalence and risk factors of *Salmonella* in commercial poultry farms in Nigeria. *PLoS One* 15 (9): e0238190. DOI: 10.1371/journal.pone.0238190.
- Kong-Ngoen T, Santajit S, Tunyong W, Pumirat P, Sookkrung N, Chaicumpa W, Indrawattana N. 2022. Antimicrobial resistance and virulence of non-typhoidal *Salmonella* from retail foods marketed in Bangkok, Thailand. *Foods* 11: 661. DOI: 10.3390/foods11050661.
- Lai J, Wu C, Wu C, Qi J, Wang Y, Wang H, Liu Y, Shen J. 2014. Serotype distribution and antibiotic resistance of *Salmonella* in food-producing animals in Shandong province of China, 2009 and 2012. *Intl J Food Microbiol* 180: 30-38. DOI: 10.1016/j.jfoodmicro.2014.03.030.
- Lettimi AA, Vo Than T, Marafin E, Longo A, Antonello K, Zavagnin P. 2016. Distribution of *Salmonella* serovars and antimicrobial susceptibility from poultry and swine farms in Central Vietnam. *Zoonoses Public Health* 63 (7): 569-576. DOI: 10.1111/zph.12265.
- Mahmud MS, Bari ML, Hossain MA. 2011. Prevalence of *Salmonella* serovars and antimicrobial resistance profiles in poultry of Savar area, Bangladesh. *Foodborne Pathog Dis* 8 (10): 1111-1118. DOI: 10.1089/fpd.2011.0917.
- Medalla F, Gu W, Mahon BE, Judd M, Folster J, Griffin PM. 2017. Estimated incidence of antimicrobial drug-resistant nontyphoidal *Salmonella* infections, United States, 2004-2012. *Emerg Infect Dis* 23 (1): 4-12. DOI: 10.3201/eid2301.160771.
- Mridha D, Uddin MN, Alam B, Akhter AT, Islam SS, Islam MS. 2020. Identification and characterization of *Salmonella* spp. from samples of broiler farms in selected districts of Bangladesh. *Vet World* 13 (2): 275. DOI: 10.14202/vetworld.2020.275-283.
- Musawa AI, Bashiru G, Al-Rasheed A, Yakubu Y, Jibril AH, Ballah FM, Sidi S, Lawal N, Bala JA, Odhah MN, Muhammad N, Umar M. 2021. Prevalence and antimicrobial susceptibility profiling of *Salmonella* isolated from poultry products sold in Sokoto metropolis, Nigeria. *J Anim Health Prod* 9 (2): 148-155. DOI: 10.17582/journal.jahp/2021/9.2.148.155.
- National Bureau of Statistics. 2012. Federal Republic of Nigeria, Annual Abstract of Statistics, 2012. www.nigerianstat.gov.ng.
- Nguyen TK, Nguyen LT, Chau TTH, Nguyen TT, Tran BN, Taniguchi T, Hayashidani H, Ly KTL. 2021. Prevalence and antibiotic resistance of *Salmonella* isolated from poultry and its environment in the Mekong Delta, Vietnam. *Vet World* 14 (12): 3216-3223. DOI: 10.14202/vetworld.2021.3216-3223.
- Odoch T, Wasteson Y, L'Abe'e-Lund T, Muwonge A, Kankya C, Nyakarahuka L. 2017. Prevalence, antimicrobial susceptibility and risk factors associated with non-typhoidal *Salmonella* on Ugandan layer hen farms. *BMC Vet Res* 13 (1): 1-10. DOI: 10.1186/s12917-016-0931-1.
- Permatasari DA, Witaningrum AM, Wibisono FJ, Effendi MH. 2020. Detection and prevalence of multidrug resistant *Klebsiella pneumoniae* strains isolated from poultry farms in Blitar, Indonesia. *Biodiversitas* 21: 4642-4647. DOI: 10.13057/biodiv/d211024.
- Pławinska-Czarnak J, Wódz K, Kizerwetter-Swida M, Bogdan J, Kwieciński P, Nowak T, Strzałkowska Z, Anusz K. 2022. Multi-drug

- resistance to *Salmonella* spp. when isolated from raw meat products. *Antibiotics* 11: 876. DOI: 10.3390/antibiotics11070876.
- Putra AR, Effendi MH, Koesdarto S, Suwarno S, Tyasningsih W, Estoepangestie AT. 2020. Detection of the extended spectrum  $\beta$ -lactamase produced by *Escherichia coli* from dairy cows by using the vitek-2 method in Tulungagung regency, Indonesia. *Iraqi J Vet Sci* 34 (1): 203-207. DOI: 10.33899/ijvs.2019.125707.1134.
- Riwu KHP, Effendi MH, Rantam FA. 2020. A review of extended-spectrum  $\beta$ -Lactamase (ESBL) producing *Klebsiella pneumoniae* and Multidrug-Resistant (MDR) on companion animals. *Syst Rev Pharm* 11 (7): 270-277. DOI: 10.31838/srp.2020.7.43.
- Robertson J, Yoshida C, Kruczkiewicz P, Nadon C, Nichani A, Taboada EN. 2018. Comprehensive assessment of the quality of *Salmonella* whole genome sequence data available in public sequence databases using the *Salmonella* in silico Typing Resource (SISTR). *Microb Genomics* 4 (2): 14-18. DOI: 10.1099/mgen.0.000151.
- Sarker BR, Ghosh S, Chowdhury S, Dutta A, Chandra Deb L, Sarker BK. 2021. Prevalence and antimicrobial susceptibility profiles of non-typhoidal *Salmonella* isolated from chickens in Rajshahi, Bangladesh. *Vet Med Sci* 7 (3): 820-830. DOI: 10.1002/vms3.440.
- Slader J, Domingue G, Jorgensen F, McAlpine K, Owen RJ, Bolton FJ, Humphrey TJ. 2002. Impact of transport crate reuse and of catching and processing on *Campylobacter* and *Salmonella* contamination of broiler chickens. *Appl Environ Microbiol* 68: 713-719. DOI: 10.1128/AEM.68.2.713-719.2002.
- Siddiky NA, Sarker S, Khan SR, Rahman T, Kafi A, Samad MA. 2022. Virulence and antimicrobial resistance profile of non-typhoidal *Salmonella enterica* serovars recovered from poultry processing environments at wet markets in Dhaka, Bangladesh. *PLoS ONE* 17 (2): e0254465. DOI: 10.1371/journal.pone.0254465.
- Sohail MN, Rathnamma D, Chandra PS, Isloor S, Naryanaswamy HD, Wilfred RS, Veeregowda BM. 2021. *Salmonella* from farm to table: Isolation, Characterization, and antimicrobial resistance of *Salmonella* from commercial broiler supply chain and its environment. *BioMed Res Intl* 2021. DOI: 10.1155/2021/3987111.
- Sin M, Yoon S, Kim YB, Noh EB, Seo KW, Lee YJ. 2020. Molecular characteristics of antimicrobial resistance determinants and integrons in *Salmonella* isolated from chicken meat in Korea. *J Appl Poult Res* 29 (2): 502-514. DOI: 10.1016/j.japr.2019.12.010.
- Useh NM, Ngbede EO, Akange N, Thomas M, Foley A, Keena MC, Nelson E, Christopher-Hennings J, Tomita M, Suzuki H, Scaria J. 2016. Draft genome sequences of 37 *Salmonella enterica* strains isolated from poultry sources in Nigeria. *Genome Announcements* 4 (3): e00315-16. DOI: 10.1128/genomeA.00315-16.
- Usha G, Chunderika M, Prashini M, Willem SA, Yusuf ES. 2008. Characterization of extended-spectrum  $\beta$ -lactamases in *Salmonella* spp. at a tertiary hospital in Durban, South Africa. *Diagn Microbiol Infect Dis* 62 (2): 86-91. DOI: 10.1016/j.diagmicrobio.2008.04.014.
- Tack B, Vanaenrode J, Jan YV, Jaan T, Jan J. 2020. Invasive non-typhoidal *Salmonella* infections in Sub-Saharan Africa: A systematic review on antimicrobial resistance and treatment. *BMC Med* 18: 212. DOI: 10.1186/s12916-020-01652-4.
- Telli AE, Biçer Y, Telli N, Güngör C, Turkal G, Onmaz NE. 2022. Pathogenic *Escherichia coli* and *Salmonella* spp. in chicken carcasses: Isolation and genotyping by ERIC-PCR. *Pak Vet J* 42 (4): 493-498. DOI: 10.29261/pakvetj/2022.049.
- Wibisono FJ, Sumiarto B, Untari T, Effendi MH, Permatasari DA, Witaningrum AM. 2020a. Short Communication: Pattern of antibiotic resistance on extended-spectrum beta-lactamases genes producing *Escherichia coli* on laying hens in Blitar, Indonesia. *Biodiversitas* 21: 4631-4635. DOI: 10.13057/biodiv/d211022.
- Wibisono FM, Wibisono FJ, Effendi MH, Plumeriastuti H, Hidayatullah AR, Hartadi EB, Sofiana ED. 2020b. A review of Salmonellosis on poultry farms: Public health importance. *Syst Rev Pharm* 11 (9): 481-486. DOI: 10.31838/srp.2020.9.69.
- Wibisono FM, Faridah HD, Wibisono FJ, Tyasningsih W, Effendi MH, Witaningrum AM, Ugbo EN. 2021. Detection of *invA* virulence gene of multidrug-resistant *Salmonella* species isolated from the cloacal swab of broiler chickens in Blitar district, East Java, Indonesia. *Vet World* 14 (12): 3126-3131. DOI: 10.14202/vetworld.2021.3126-3131.
- Wibisono FJ, Rahmani RP, Syaputra DE, Zuriya Z, Aziz KM, Ikeng LD, Effendi MH, Bernard AN. 2023. Risk factors for non-typhoidal *Salmonella* contamination in chicken meat: A cross-sectional study on traditional markets in Surabaya. *Adv Life Sci* 10 (2): 282-288.
- Yanestria SM, Rahmani RP, Wibisono FJ, Effendi MH. 2019. Detection of *invA* gene of *Salmonella* from milkfish (*Chanos chanos*) at Sidoarjo wet fish market, Indonesia, using polymerase chain reaction technique. *Vet World* 12 (1): 170-175. DOI: 10.14202/vetworld.2019.170-175.
- Yang X, Wu Q, Zhang J, Huang J, Chen L, Wu S, Zheng H, Wang J, Chen M, Wu H, Gu Q, Wei X. 2019. Prevalence, bacterial load, and antimicrobial resistance of *Salmonella* serovars isolated from retail meat and meat products in China. *Front Microbiol* 10: 2121. DOI: 10.3389/fmicb.2019.02121.