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A Review of Poultry Product as a Source of Spreading Multidrug Resistant Salmonella: Public Health Importance

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ABSTRACT

In the last few decades, foodborne disease has become one of the world's health problems with various pathogenic bacteria that accompanies the contamination of food products of animal origin. One of the foodborne diseases that is always reported every year is related to Salmonella contamination in poultry products which can cause Salmonellosis in humans. Salmonella contamination become important not because of its virulence ability to invade humans, but also because of its increased resistance to various clinical antimicrobial classes, with various cellular genetic elements that can be spread in humans along the food chain. The purpose of this review is to provide an overview of the role of poultry product in the spread of multidrug resistance Salmonella which may have implications for public health.

Keyword: Foodborne Disease; Salmonella; Virulence Factor; Multidrug-Resistance; Mobile Genetic Element

INTRODUCTION

Foodborne disease has become one of the global public health problems lately, given its implications for health and the economy. Various kinds of pathogens play a role in foodborne disease (Ejo*et al.*, 2016). It is estimated that 17.9% of all foodborne diseases related to poultry and 19% of foodborne diseases associated with poultry are caused by *Salmonella enterica* contamination and infection O'Bryan *et al.,* 2022).Foodborne disease caused by Salmonella infection, distributed in animals and foodstuffs of animal origin (Pires and Hald, 2010) and considered as the main carrier for humans(Ejo et al, 2016). In various parts of the world, foodborne disease caused by *Salmonellosis*, causes an increase in invasive disease, hospitalization and death (Pires and Hald, 2010) which can have a significant effect on children, the

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elderly and immunocompromised (Ding and Fu, 2016). Globally, there are 94 million cases of gastroenteritis and 155,000 deaths caused by Salmonella infection each year (Yang et all, 2019).

Salmonella contamination occurs through consumption of contaminated food such as eggs, milk and poultry meat. Twenty percent of the world's poultry products are contaminated with Salmonella and the bacteria can persist for a long time in the environment, animal facilities and through biofilm formation. In most outbreaks of Salmonellosis due to consumption of poultry products, it is known that *S.* Enteritidis and *S.* Typhimurium are the most isolated serovar (Afshari et all, 2018). Infections caused by Non-Typhoid Salmonella (NTS), particularly *S.* Enteritidis and*S.* Typhimuriumare the most commonly reported infections associated with Salmonellosis in humans (Yang et all, 2019). *S.* Enteritidis and*S.* Typhimuriumare pathogenic because of their ability to invade, replicate and survive in human host cells (Sodagari et al, 2020). The pathogenesis of Salmonella and its interaction with the host depends on several virulence factors encoded by many genes distributed on chromosomes and plasmids (Borges et al, 2019).

At the same time, antibiotic resistance in *S.* Enteritidisand *S.* Typhimurium has also become one of the most important public health problems worldwide. The emergence of resistance to broad-spectrum cephalosporins and fluoroquinolones is of great public health importance, considering that this class of antibiotics is critical for the management of human salmonellosis cases (Yang et al, 2019). This paper will review the role of poultry and their products that can act as a factor in the spread of multidrug resistant (MDR)*Non-Typhoid Salmonella* and aspects related to food safety with One Health approach to understand the impact on public health, animals, and the environment.

DISCUSSION

Salmonella

Bacteria from the genus Salmonella belong to the family *Entrobacteriaceae*, Gram negative, facultative anaerobic, non-producing spores, have petrichous flagella and motile (Cosby et all, 2015), except for*S.* Gallinarumand *S.* Pullorum (Jajere, 2019). Salmonella is able to reduce nitrate to nitrite, well grown at of 35–40 (Cosby et all, 2015), can metabolize nutrients chemoorganotrophically and unable to ferment lactose. Salmonella has the broadest predilection for the digestive tract of humans and animals. Salmonella is divided into 2 groups of species, *Salmonella enterica* and *Salmonella bongori*, based on the differences in their 16S rRNA. Based on biochemical properties and genomic linkages, *Salmonella enterica* was classified into six subspecies (*S.*

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*enterica*subsp. *enterica,S. enterica* subsp.*S. salamae,S. enterica* subsp. *arizonae, S. enterica* subsp. *diarizonae, S. enterica* subsp. *houtenae and S. enterica* subsp.*indica*) (Jajere, 2019).

Through the Kauffman-White scheme, Salmonella is classified based on antigenic differences, namely flagellar (H), capsular (K) and somatic (O) (Heredia and García, 2018). *S. enterica*subsp. *enterica*is responsible for more than 99% of cases of human *Salmonellosis*, of which 1,531 serotypes are *S.* Typhimurium and *S.* Enteritidis (Heredia and García, 2018). *Non-Typhoid Salmonella*are zoonotic and are generalist hosts that can infect various warm-blooded animals, including humans (Arya et all, 2017). Salmonella serotypes, such as Enteritidis, Typhimurium, Newport, Heidelberg and Montevideo are known to contribute in Salmonellosis through their contamination of various food products including chicken, pork, eggs, vegetables and milk (Andino and Hanning, 2015). The distribution of Salmonella serotypes in various food products shown in table 1.

Table 1. The distribution of *Salmonella* serotypes in various food products

Serotype	Food Sources	References
S. Enteritidis	Poultry Meat, Egg, Retail Ground Turkey, Seafood	Carrasco *et al.*, 2012; Yang *et al.*, 2015
S. Dublin	Minced Beef, Chicken Meat, Goat and Camel Meat	Molla*et al.*, 2003; Tadesse and Gebremedhin, 2015
S. *Infantis*	Organic Raw Chicken, Pork	Rajan*et al.*, 2016; Simpson *et al.*, 2018
S. Montevideo	Beef	Ferrari *et al.*, 2019
S. Typhimurium	Chicken Meat, Sausages, Seafood, Watermelon, Pork, Dairy, Beef	Jarquin*et al.*, 2015; Bouchrif*et al.*, 2009; D'Aoust, 1994; Simpson *et al.*, 2018
S. Heidelberg	Retail Ground Chicken	Rajan*et al.*, 2016; Jarquin*et al.*, 2015
S. Javiana	Tomato	D'Aoust, 1994
S. Hadar	Pork, Frozen Chicken, Duck and Seafood	Ferrari *et al.*, 2019; Bangtrakulnonth*et al.*, 2004
S. Virchouw	Seafood, Egg	Simpson *et al.*, 2018
S. Derby	Pork	Ferrari *et al.*, 2019

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S. Newport	Beef, Goat and Camel Meat	Tadesse and Gebremedhin, 2015

Virulence Factors

Type III Scretion System (T3SS)

The main characteristics of virulence and factors in *S. enterica* serovars such as invasion or intracellular replication in host cells. These factors include flagella, capsule, plasmid, adhesion system and Type III Secretion System (T3SS) (Hassena et al, 2021). One of the major genetic elements, namely Salmonella Pathogenicity Island-1 (SPI1), determines the virulence ability among Salmonella serotypes (Lostroh and Lee, 2001). Enteropathogenic bacteria, including *S. enterica* have a type III secretion system (T3SS) which plays an important role in their virulence ability. This system allows the translocation of bacterial virulence proteins into the host cell cytosol (Akopyan et al., 2011)(Park et al., 2018). These proteins are known as effectors, which can modulate or interfere with various host cellular processes (Sun et al., 2016), facilitating bacterial colonization and survival (Feria et al., 2015). The central element of T3SS is the injectisome, a multi-protein machinery (Park et al., 2018) consisting of a needle complex (de Souza Santos and Orth, 2019). T3SS consists of a cylindrical basal body ~26 nm in diameter and ~32 nm in height (Kato et al., 2018), with a two-ring structure encompassing the bacterial inner and outer membranes (de Souza Santos and Orth, 2019). As well as the cytoplasmic structure that is used to sort effector proteins (Kato et al., 2018) and provide energy for the secretory process (Hu et al., 2017). T3SS measures about 3.5 MDa spanning the double membrane and protruding into the extracellular space. About 25 structural proteins and additional proteins are required for their assembly (Puhar et al., 2014).

Salmonella Pathogenicity Island (SPI)

The virulence factor of *S. enterica*is encoded by a conserved gene on Salmonella Pathogenicity Island (SPI) (Askoura and Hegazy, 2020). The existence of this SPI is obtained horizontally (Eade et al., 2019) which occurs through conjugation, transformation and transduction mechanisms (Pradhan and Negi, 2019) (Zishiri et all, 2016). There are five main SPIs (1-5) (Lamas et all, 2018), of which SPI-1 and SPI-2 contain a large number of virulence genes related to intracellular pathogenesis and co-encode T3SS (Wang et all, 2020). SPI-1 is 40-kb in size, which includes 39 genes encoding T3SS-1, their chaperone and effector proteins. As well as several transcriptional regulators that control the expression of virulence genes inside and outside SPI-1 (Lou et all, 2019). The expression level of the SPI-1 gene is dependent on the HilA regulator encoded SPI-1, which directly activates the expression of the SPI-1 structural gene (Golubeva et al., 2016). SPI-1 T3SS is expressed by Salmonella in the early stages of infection, which can stimulate inflammation (Kim et al., 2018). In contrast, SPI-2 is required by Salmonella for growth in different host cells (Dhanani et all, 2015) (Jennings et all, 2017), including macrophages (Fardsanei et all, 2017). SPI-3 is used by Salmonella in the process of intracellular proliferation and Mg2+ uptake and systemic spread. And SPI-5 which plays an important role in the development of the infection process and intracellular survival (Bertelloni et al., 2017).

Virulence Plasmid

Non-Typhoidal Salmonella also carries a virulence plasmid (Dos Santos et all, 2019). Salmonella virulence plasmids are 50–90 kb in size with a low copy number (1-2 plasmids per chromosome) that can be transmitted (Lobato-Márquez, 2016). In *S.* Typhimurium, it has a size of ~90 kb (pSLT) (Passaris et all, 2018) with an 8 kb region and a highly converted gene sequence, termed the Salmonella plasmid virulence (spv) locus (Silva et al., 2017) and functions as serum resistance, adhesion, colonization and promote the growth and reproduction of bacteria in host cells and tissues (Wu et all, 2016). Virulence plasmids also encode genes required for systemic infection (Abraham et all, 2018), such as the pef gene (plasmid encoded fimbriae), which plays a role in adhesion to crypt epithelial cells and induction of proinflammatory responses (Silva et al., 2017); the spv gene which is used to suppress the host's innate immune response (Abraham et al., 2018) and the rck gene which is used to develop resistance capabilities to the host's innate immune complement system (Cheng et al., 2019) (Dos Santos et al., 2019).

Fimbriae

Through different virulence factors, Salmonella also develops an adhesin function on the fimbriae which are used to attach to host cells (Rehman et al, 2019). Salmonella uses its fimbriae through interaction with proteins on host cell receptors, to be able to carry out adhesion and colonization in the intestine (Hansmeier et al, 2017). Fimbriae are generally 0.5-10 nm long and 2-8 nm wide (Rehman et all, 2019). Fimbriae in Salmonella are generally grouped into classes according to their assembly mechanism, namely (1) curli fimbriae which are assembled through a nucleation-precipitation (N/P) process through deposition of the main subunit with the extracellular media nucleator (Dufresne et al., 2018); (2) chaperone-usher (CU) fimbriae are assembled using periplasmic chaperones and usher outer periplasmic membranes, to form the main subunit into the final external filament (Rehman et all, 2019) and (3) type IV fimbriae are assembled on the inner and outer membranes.

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extended through the periplasm and outer membrane to the extracellular environment. These fimbriae can be assembled or disassembled using ATP (Dufresne et al., 2018). However, fimbriae in *S*. Enteritidishave different structures, these fimbriae are assembled using the CU system and consist of several subunits (Quan et al., 2019). In addition, it is also classified based on different clades, γ, κ, π, β, α and σ (Rehman et all, 2019). Among these clades, the lpf and fim genes belong to subclade γ1, sef subclade γ3, pef subclade κ and sdc in subclade σ(Quan et al., 2019).

Flagella

Bacterial motility comes from organelles called flagella. More than 40 genes are responsible for flagella assembly and its motor function (Kubori et al., 1992). In each strain of Salmonella, each has flagella of different types of H-antigens, with different primary structures (Asakura et al., 1966). Flagella are morphologically divided into three parts: filaments, hooks and basal structures (Aizawa, 1996). The basal body is an important part of flagella motor function (Kubori et al., 1992) and is morphologically divided into an inner membrane ring (MS), a rod and an outer ring (LP) (Jones and Macnab, 1990) which are embedded in the outer membrane. then extends into the periplasmic space. The MS ring structure is considered as the rotor, the rod as the shaft and the LP ring as the bushings. (Kubori et al., 1992). Filaments and hooks are on the outside of the cell (Aizawa, 1996), while the basal structure is anchored on the outer and inner membranes (Kawamoto et al., 2013). In Salmonella, the genes responsible for flagella formation are about 50 genes grouped into 17 operons, where each operon is divided into three classes according to the order of expression (Alzawa, 1996). The gene is flg, flh, fli or flj. While the genes responsible for flagella function are encoded by flagellar rotation (mot), chemotaxis (che) genes, and transmembrane signal transduction of chemotactic stimuli (tar, trg, tsr, etc.) (Kutsukake et al., 1990). Distribution of Virulence Factors among *Salmonella* Species is presented in table 2.

Table 2. Distribution of virulence factors among *Salmonellosis* species

Virulence Factor	Location	Salmonella species	Function	Reference
hilA	Chromosome (SPI-1)	*S. enterica*	Transcriptional regulation of the genes of SPI-1, Modulation of colonization and invasion	Bottledoorn*et al.*, 2010; Song *et al.*, 2016; Cheng *et al.*, 2019
invA			Invasive effector protein, production of proteins in T3SS	Cheng *et al.*, 2019; Awad*et al.*, 2020
sip			Cytoskeleton rearrangement, Membrane ruffling, Invasion	Cheng *et al.*, 2019; Dos Santos *et al.*, 2019
sop			Colonization and induces remodeling of actin	Ilyas *et al.*, 2017; Cheng *et al.*, 2019; Wang *et al.*, 2020
sif	Chromosome (SPI-2)		Invasion effector, NLRC4 inflammasome, SCV membrane stability, t3SS1-independent inflammation factor, luminal colonization	Beshiru*et al.*, 2019; Wang *et al.*, 2020; Hyeon *et al.*, 2021
sspH	Chromosome (SPI-12)	*S.* Enteritidis, *S.* Typhimurium	Intracellular replication	Suez *et al.*, 2013; Shapo *et al.*, 2020
sef	Chromosome	*S.* Enteritidis, *S.* Gallinarum, *S.* Pullorum, *S.* Typhi, *S.* Dublin,	Mediating attachment in host cells, Uptake or survival in macrophages	Rank *et al.*, 2009; Bottledoorn*et al.*, 2010; Hu *et al.*, 2019
pef	Plasmid (pSLT)	*S.* Enteritidis, *S.* Typhimurium	Adhesion to crypt epithelial cells, biofilm growth, induction of a pro-inflammatory response	Silva *et al.*, 2017; Quan *et al.*, 2019; Awad*et al.*, 2020

spv	Plasmid (pSLT)	*S.* Enteritidis, *S.* Typhimurium, *S.* Cholerasuis, *S.* Dublin	induces apoptosis of macrophages, MAPK inactivation, Survival and growth in the macrophage	Bottledoorn*et al.*, 2010; Suez *et al.*, 2013; Tasmin *et al.*, 2019
fliC	Chromosome	*S. enterica*	Adhesion and invasion, immune modulation, proinflammatory effect	Herrera-Leon *et al.*, 2004; Liu *et al.*, 2017; Magdy *et al.*, 2020

TRANSMISSION

Poultry products are widely consumed globally where the presence of Salmonella bacteria is also found in live poultry, poultry environment, retail meat and meat products. *S. enterica*subsp. *enterica* can be transmitted to humans in different ways. On farms, Salmonella is often excreted in the feces. This allows for fecal-oral transmission. In addition, Salmonella vertical transmission occurs from chicken to egg. Therefore, humans can be contaminated through consumption of contaminated eggs (Monte et al, 2019). Environmental contamination with low antimicrobial levels can lead to an increase and persistence of the population of resistant bacteria. In addition, exposure of bacterial populations to antimicrobials can also result in the exchange of resistant genetic elements, which may include virulence genes (Monte et al, 2019). In addition, nosocomial transmission has also been found between animals of different species, which has implications for the ability of NTS serovars to survive and move from different environmental sources to susceptible hosts (Cheng et all, 2019). Transmission among *Salmonella* species in poultry products shown in table 3.

Table 3. Transmission and contamination among *Salmonella* species in Poultry Products

Transmission Routes	Sources of Contamination	References
Horizontal	Feed and drinking water contamination, rodents and insects, environments, poor level of hygiene	Heydrickx*et al.*, 2002; Macirowski*et al.*, 2004; Rasschaert*et al.*, 2008
Vertical	Trans-ovarian	Van de Giessen *et al.*, 1994;

		EFSA BIOHAZ Panel *et al.*, 2019
Human Traffic	Employee movement between different farms and contact with different species of animals	Nair *et al.*, 2019; Zeng *et al.*, 2021
Poultry meat production chain	. Scalding, defeathering, evisceration, chilling	Nauta*et al.*, 2000; Nair *et al.*, 2019
Food Product	Contact surfaces, utensils and environmental, Retail chicken meat contamination	Nidaullah*et al.*, 2017; Shang *et al.*, 2019

SALMONELLA CONTAMINATION IN POULTRY PRODUCTS

Various foodborne pathogens have been reported to be associated with consumption of poultry products (meat and eggs) (Lukicheva et all, 2016) (Zhang et all, 2021) caused by Non-Typhoid Salmonella (NTS) contamination (Chousalkar and Gole, 2016). Poultry has been considered as the main cause of *Salmonellosis*in humans through consumption of contaminated meat and eggs (Siddiky et all, 2021) (Toro et all, 2016). Poultry, especially chickens, are often colonized by *Salmonella* without symptoms (subclinical infection) (Antunes et al, 2016). Salmonella can enter and survive in the farm environment for long periods of time. According to recent research, it is known that the prevalence of Salmonella in the livestock environment ranges from 10 to 26% (Andino and Hanning, 2015). Infected poultry flocks also play a role as a reservoir for Salmonella which can be transmitted through the food chain (Saravanan et all, 2015) (Varmuzova et all, 2016). Poultry generally carry Non-Typhoid Salmonella (NTS) such as *S.* Enteritidisand *S.* Typhimurium(Wang et all, 2020) (Sarker et all, 2021).

S. Enteritidis is known to be very well adapted to the cage and egg environment. Salmonella infection in poultry is often caused by *S.* Enteritidis which transmits vertically and trans-ovarianly. In addition, contamination caused by *S.* Typhimurium and other serovars occurs externally by penetrating the egg shell (Andino and Hanning, 2015). In addition, the surface of chicken meat can be contaminated with Salmonella from intestinal contents, faecal material or from cross-contamination during the slaughter process (da Cunha-Neto et al, 2018). (Banggera et all, 2019). In the few cases of salmonellosis outbreaks that occurred in Australia, the United States and the United Kingdom, a large number of outbreaks of gastrointestinal infections due to foodborne disease were associated with eggs. The pattern of consumption of raw or undercooked eggs is often associated with cases of salmonellosis. *S.*Enteritidis is a major concern for most of the poultry industry (Chousalkar and Gole, 2016).

Through a number of studies in various countries, the prevalence of Non-Typhoid Salmonella (NTS) contamination in chicken meat in the Hanoi area, Vietnam is 71.8% with the highest percentage of contamination occurring in traditional markets (90%) compared to supermarkets (52.6%). (Nhung et al, 2018). 14.89% of chicken meat in Northern India, much higher than the 7.01% poultry faeces sample (Sharma et all, 2019) and 63.6% in chicken meat in traditional markets in Guangdong region, China (Zhang et al., 2018) and 26.70% in the Malaysian region (Thung et all, 2016). Contamination among *Salmonella* species in poultry products shown in table 3.

MULTIDRUG RESISTANT SALMONELLA

Antibiotic resistance is a global phenomenon that results in the emergence of pathogens with resistance to clinically important antibiotics, thus requiring new treatment strategies (Nair et al, 2018). During the last few decades, there has been a global increase in the widespread and excessive use of antimicrobial agents. Both in humans and animals (Ammar et all, 2018) (Castro-Vargas et all, 2019). which contributes to the spread of antibiotic resistance (AMR) among Salmonella serotypes (Lenchenko et all, 2020) (Langata et all, 2019). This has resulted in an increase in the frequency of Salmonella serotypes which then develop their resistance ability to become multidrug resistant (MDR) which can cause new problems for human and animal health (Levantesi et al., 2012) (Siddiky et al., 2022). Several studies have shown that multidrug resistance among Salmonella serotypes was most frequently observed in ASSuT (ampicillin, streptomycin, sulfonamide and tetracycline), ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfonamide and tetracycline) (Nair et all, 2018) (Xie et all, 2019) (Xiang et al., 2020). Also, fluoroquinolones, Extended Spectrum Beta-Lactamase (ESBL) (Aldrich et all, 2019), Quinolone (Abatcha et all, 2018) and Ciprofloxacin (González and Araque, 2019) (Harb et all, 2018).

Antimicrobial resistance in Salmonella can be intrinsic, acquired or adaptive (Christaki*et al.,* 2020).The intrinsic resistance mechanism occurs through decreased permeability of the outer membrane (lipopolysaccharide) and the natural activity of efflux pumps (Reygaert, 2018). Antimicrobial resistance is acquired when a susceptible strain becomes resistant as a consequence of the evolution of a new strain, as a result of a mutation in a bacterial population or the acquisition of a specific resistance gene through horizontal gene transfer (HGT) (Verraes*et al.,* 2013).Adaptive resistance occurs because the modulation results in gene expression in response to environmental changes (stress, growth state, pH, ionsconcentrations, nutrient conditions and sub-inhibitory levels of antibiotics) (Christaki*et al.,* 2020). Multidrug resistance*Salmonella* isolates adapted to various resistance mechanisms i.e., modification of drug target sites, production of drug-degrading enzymes and overexpression of efflux pumps (Anbazhagan*et al.,* 2019).

The modification of the drug target site, occurs through alteration of the *Penicillin-Binding Protein* (PBP) in β-lactam, which causes a change in the amount of antimicrobial that can bind to the PBP (Reygaert, 2018).In addition, quinolone resistance occurs as a result of chromosomal mutations in *gyrA*and *parC*, subunit of DNA gyrase andtopoisomerase IV (Christaki*et al.,* 2020) (Eichenberger and Thaden, 2019).Resistance to drugs that target the ribosomal subunit occurs through ribosomal mutations (aminoglycosides, oxazolidinones) in the *erm* gene. In addition, mutations in the enzymes *dihydropteroate synthase* (DHPS, Sulfonamide) and *dihydrofolate reductase* (DHFR, Trimethoprim) causing structural changes in the binding site with antimicrobial agents (Reygaert, 2018). Multidrug efflux systems are almost invariably encoded by chromosomal genes that are expressed either intrinsically or acquiredly (Poole, 2007) and occur on mobile genetic elements (transposons, integrons, plasmids) (Ruppé*et al.*, 2015) whose acquisition from otherresistance organisms (Poole, 2007) which capable of dispensing a wide variety of structurally different compounds (Arzanliu*et al.,* 2017). Modifying enzymes may be plasmid mediated or chromosomal (Sefton, 2002).β-lactamases are the best example of antibiotic resistancemediated by modifying enzymes mechanism (Christaki*et al.,* 2020) and can be acquired via horizontal gene transfer (Eichenberger and Thaden, 2019). Multidrug resistance mechanisms summarized in table 4.

Table 4. Multidrug Resistance Mechanism

Mechanisms of Action	Antimicrobial Agents
Efflux Systems	Tetracyclines β-lactam
Mutation	Sulfonamide Trimethoprim Aminoglycosides Oxazolidinones
Modification of the drug target site	Fluoroquinolone β-lactam Quinolone

Mobile Genetic Element – Resistance Plasmid

Multidrug resistant Salmonella is transferred from food of animal origin through the food chain by carrying different Antibiotic Resistance Genes (ARGs) (Liu et al, 2020). Commensal bacteria and pathogens in the gut can exchange cellular genetic elements that mediate resistance (Maka and Popowska, 2016). ARGs carried by Salmonella are located on plasmids, chromosomes, transposons and transferable integrons by cellular genetic elements (MGE) (Ramatla et all, 2019) (Aziz et all, 2018) (Penha Filho et all, 19). DNA fragments encoded in cellular genetic elements can recombine with bacterial chromosomes or with other elements present in bacterial cells (Brown-Jaque et al., 2015). Plasmids are circular DNA extra-chromosomal elements capable of replicating independently of the host genome (Mathers et al., 2015). Resistance plasmids are generally conjugative and can be mobilized (Sultan et al., 2018). Through the mechanism of conjugation, plasmids are transferred from the donor to the recipient cell, through contact-dependent transmission and an energy-driven process (Carattoli, 2011). Antimicrobial resistance plasmids were divided into 2 main groups, namely (1) narrow host group, incompatibility F(*IncF*) and (2) broad host group, IncA/C, IncL/M, and IncN (Mathers et al., 2015). The IncF plasmid in Salmonella is known to carry the genes blaTEM-1 (β-lactam), *cmlA* (chloramphenicol) and integrons with the genes *drfA* (trimethoprim), *aadA1, aadA2* (aminoglycosides) and *sul3* (sulfonamides) (Garcia et al., 2014). Meanwhile, the IncA/A plasmid carried 10 AMR genes for more than five antimicrobial classes, such as *straAB* (aminoglycoside), *sul2* (sulfonamide), *tetAR* (tetracycline), *blaCMY-2* (β-lactam) and *floR* (chloramphenicol) (McMillan et al.., 2020). Also, other genes encoding resistance to trimethoprim and cephalosporines (McMillan et al., 2019).

Mobile Genetic Element – Transposons and Insertion Sequences

Transposons (TN) are transposable elements that include small cryptic elements or insertion sequences (IS), transposons and transposition bacteriophages that facilitate the movement of DNA fragments from one location to another on bacterial chromosomes and plasmids (Tripathi and Tripathi, 2017) (Partridge et al., 2018). Insertion sequences (IS) are sandwiched between short, inverted and repeating sequences flanking the coding region of the gene (Brown-Jaque et al., 2015). From 10-40 base pairs at both ends (Sultan et al., 2018). The entire DNA fragment from one IS element to another is transposed as a complete unit (Brown-Jaque et al., 2015). Insertion sequences (IS) are classified according to different nuclease catalytic domains, namely DD (E/D), HUH, phosphoserine and phosphotyrosine site-specific recombinase, which can be found in transportase, invertase or resolvase (Razavi et al., 2020). Meanwhile,

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transposons are categorized into two classes, namely (1) composite transposons (carrying various resistance genes that have identical structural and functional characteristics, with small DNA homology) and (2) complex transposons (three distinct but related families; Tn3, Tn21 and Tn2501 (Sultan et al., 2018). Transposons can be transmitted depending on transposition proteins (reverse transcriptase enzymes) i.e., autonomously (*pol, gag* and open reading frame or *ORF* genes in them) (Babakhani and Oloomi, 2018) or non-autonomously, which requires the presence of other transposons to move (Babakhani and Oloomi, 2018) Brown-Jaque et al., 2015). Several transposons associated with antimicrobial resistance include Tn5, Tn10 (kanamycin, neomycin, tetracycline) and Tn21 (streptomycin, spectinomycin, sulfonamide) (Tripathi and Tripathi, 2017).

Mobile Genetic Element – Integron

Integrons are mobile DNA elements consisting of site-specific recombination systems (Meng et al., 2017) that are capable of integrating, assembling and expressing resistance-related genes in the gene cassette structure (Tripathi and Tripathi, 2017). As well as transferring from one bacterium to another (Meng et al., 2017). In general, integrons have structures in the form of (1) *intl* genes encoding site-specific tyrosine recombinase enzyme (Kaushik et al., 2018) which facilitate gene transfer by sequential incorporation of genes at the attI recombination site; (2) the *attI* recombination site integrated with the gene cassette, located upstream of the *intI* gene (Escudero et al., 2015); and (3) the promoter (*Pc*) that regulates the expression of the captured gene (Pereira et al., 2020), resides within the intl gene (between the *intI* and *attI* sites) (Escudero et al., 2015) that is oriented to the integration point (Kaushik et al., 2018). Integran-mediated genes are regulated by gene cassettes. each cassette consists of an open reading frame (*ORF*) together with an attC recombination site (Pereira et al., 2020). Gene cassettes are relatively small (500–1000bp) (Escudero et al., 2015), can be free-loop, are non-replicative and are found to be inserted inside the integron (Partridge et al., 2018). Integrons are grouped into five classes based on gene integration (*Intl*) namely classes 1, 2, 3, 4 and 5 (Kaushik et al., 2018). Class 1, 2 and 3 integrons can be found on mobile genetic cellular integrons, while class 4 integrons are found on chromosomal integrons (Sultan et al., 2018). Class 1 integrons were most frequently detected in terms of antimicrobial resistance (Ma et al., 2017) with linkage to the Tn402 transposon (Kaushik et al., 2018). Class I integrons with a high detection frequency were also found among multidrug resistant Salmonella which have conserved regions (5'-CS and 3'-CS) with a gene cassette in them (Gharieb et al., 2015). Integrons are known to play an important role in the spread of

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antimicrobial resistance Salmonella (McMillan *et al.*, 2019) (Begum et al, 2016) (Lamas et al, 2016) through site-specific recombination to transfer resistance genes between defined sites (Partridge et al., 2018). Class I integrons carry *aadA* resistance determinants (streptomycin-spectomycin), trimethoprim (Sultan *et al.*, 2018). Multidrug resistance mechanism mediated by horizontal gene transfer presented in table 5.

Table 5. Multidrug resistance mediated by mobile genetic elements (MGE)

Mobile Genetic Elements	Gene	Antimicrobial Agents
Plasmid Resistance	blaTEM-1 blaCMY-2	β-lactam
	drfA	Trimethoprim
	sul2 *sul3*	Sulfonamide
	straAB	Aminoglycoside
	tetAR	Tetracycline
	floR	Chloramphenicol
Transposon (TN)& Insertion Sequences (IS)	*Tn5, Tn10*	Kanamycin Neomycin Tetracycline
	Tn21	Streptomycin Spectinomycin sulfonamide
Integron	*attI* *Intl*	Most of antimicrobial agents
	aadA	Streptomycin-Spectomycin Trimethoprim

MULTIDRUG RESISTANT SALMONELLA IMPLICATTIONS ON HUMAN HEALTH

Antimicrobial resistance is considered as one of the main threats to human health, as well as a major concern for food safety, which in its transmission involves the food chain (Tollefson and Miller, 2000). Antibiotics are used in animal food production to promote growth, prevent (prophylaxis), treat (therapeutic), and control (metaphylaxis) infectious diseases (Bengtsson and Greko, 2014). The extensive use of antibiotics in animal production systems (Nair et al., 2018) has contributed to increased selection pressure on the emergence and spread of multidrug resistance Salmonella isolates (Parisi et al., 2018). Most human infections by MDR Non-typhoidal Salmonella (NTS) are generally of foodborne origin, with animals as reservoirs of resistance and retail meat acting as carriers of human disease (Glenn et al., 2013). The presence of antibiotics in food consumed by humans has its own implications for the development of antibiotic resistance by the human gut microbiome (Lekshmi et al, 2017). The complex route of transmission between farm animals, humans and transfer of AMR genes between bacteria makes the reservoir of AMR genes in livestock poses risks to animal and human health, considering that some of these resistant ones are zoonotic (Argudin et al, 2017). Increased antimicrobial resistance in Salmonella sp. as foodborne bacteria contribute to increasing human health consequences, such as increasing cases of foodborne disease and increasing number of treatment failures (Anderson *et al.*, 2003). Antibiotic resistance in Salmonella is strongly influenced by strains:*S.* Enteritidis, *S.* Typhimurium, *S.* Typhimurium monophasic, *S.* Infantis and *S.* Derby, where all five can be found in humans and food products such as poultry meat and eggs (Peruzy*et al.*, 2020).

RISK FACTORS OF MULTIDRUG RESISTANNCE SALMONELLA

In recent years, the risk factors associated with multidrug resistance *Salmonella* isolates have received considerable attention (Hoelzer*et al.*, 2010) and have been conducted around the world. Risk factors associated with salmonella multidrug resistance at farm include biosecurity management practices (Donado-Goody *et al.*, 2012) and antimicrobial usage (Farzan *et al.*, 2010). Season (Vico*et al.*, 2020), cage system (Taddese*et al.*, 2019),bird type flock size, downtime, environment (Odoch*et al.*, 2017) disinfection (Queslati*et al.*, 2022) and waste management (Jibril *et al.*, 2020).

Meat consumption and contact in farm environment are also important risk factors for humans (Hoelzer*el.*, 2010). Food contact with surfaces, chicken slaughtered process and hygiene practices in wet market (Moe *et al.*, 2017). Low or higher temperature during broiler transportation to the slaughterhouse (Arsenault *et al.*, 2007) have been associated with the risk factors in chicken carcasses.

CONTROL AND PREVENTION

The application of Good Farming Practices (GFPs), Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs) is very important as a preventive measure against contamination caused by

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Salmonella spp. from producers to consumers (Camino Feltes et al, 2017). To reduce the risk of AMR, surveillance of resistance in humans and foods of animal origin is important to measure the long-term effectiveness of any control measures. An integrated surveillance system helps measure and compare the prevalence rate of antibiotic resistance in the food chain (Thapa et all, 2019). For the poultry industry, it is very important to control Salmonella related to food safety, such as (i) this zoonotic agent can cause foodborne disease which has a negative impact on public health; (ii) Salmonella is important in terms of antimicrobial resistance; (iii), these bacteria can cause international restrictions on the import and export of chickens and eggs; and (iv) can reduce the health level of poultry (Pulido-Landínez, 2019). At the hatchery level, disinfection of eggs with chemicals, ozone, UV irradiation, electrostatic charging, pulsed light and plasma gas is known to prevent Salmonella contamination. Not only that, passive and active immune response-based strategies, feed modification and feed management can reduce the susceptibility of poultry to infections caused by Salmonella (Dar et all, 2017). To reduce the spread of antibiotic resistance through the food chain and the environment, the use of antibiotics must be carried out effectively, through: (i) limiting antibiotics to only therapeutic uses; (ii) ensure accurate disease diagnosis; (iii) using appropriate antibiotic agents; (iv) use of appropriate dosage and duration of treatment; (v) prohibit the use of antibiotics as growth promoters; and (vi) the use of antibiotics based on a veterinarian's prescription (Sarter et all, 2015).

CONCLUSIONS

Foodborne disease caused by Salmonella contamination in poultry products (meat and eggs) has consequences for public health problems. The pathogenicity of Salmonella is controlled by various virulence genes found on chromosomes and plasmids, which affect attachment to host cells, invasion and replication in the host body and toxin production. In addition, poultry products have been considered to be a major source of multidrug resistant (MDR) Salmonella contamination which is influenced by genes related to virulence and antimicrobial resistance (AMR) related to the potential virulence of bacteria. The increase in the number of antimicrobial-resistant Salmonella strains has become a significant public health problem. Increased risk factors and rates of multidrug resistance *Salmonella* contamination has an impact on increasing public health problems and the risk of death from bacteremia which requires the integration of housing biosecurity, hygienic slaughter practices and good food product

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processing, to ensure food safety from farm to table.

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