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OCCURRENCE AND ANTIMICROBIAL RESISTANCE OF SALMONELLAE ISOLATED FROM BROILER'S LIVER AND WASHING WATER OBTAINED FROM SMALL-SCALE POULTRY PROCESSING PLANTS

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ABSTRACT

In this investigation, fifty-five chicken livers and washing water samples were gathered from the small-scale poultry processing plants in Cairo and Giza governorate, Egypt. *Salmonella* occurrence was detected, identified, and finally phenotypically characterized for the most common antibiotic groups to detect its antibiotic resistance profiles. *Salmonella* species have been recovered from 1 out of 45 (2.2%) examined chicken livers. This isolate was biochemically identified and molecularly verified such as *Salmonella* by the *invA* gene detection using PCR. The *Salmonella* serovar was recognized as *S*. Anatum. The isolated *S*. Anatum showed resistance to ten antimicrobial agents among six antimicrobial classes, so this isolate was classified as MDR. Its resistance was against gentamicin, ampicillin, ciprofloxacin, tetracycline, chloramphenicol, cefepime, ceftazidime, ceftriaxone, cefotaxime, and aztreonam. In conclusion, chicken livers were contaminated with MDR *Salmonella* serovars, which could be extremely dangerous for human health. To control such food poisoning hazards, the necessity to implement food safety systems is imperative. Additionally, continuous updating of the occurrence and antibiotic resistance profile regarding *Salmonella* is an important food safety issue.

Keywords: S. Anatum; invA gene; chicken livers; washing water; MDR; Cefotaxime.

INTRODUCTION

Salmonella infections in chicken flocks can result in acute and chronic clinical disorders, but in recent years, their connection to human sickness outbreaks through contaminated food has garnered more attention on a global scale

(Gast et al., 2020). Non-typhoidal type of Salmonella is the first and most popular food-borne infection and a serious hazard to public health, resulting in 78.7 million illness cases and 59,000 fatalities each year (Majowicz et al., 2010). S. Enteritidis and S. Typhimurium are among the majority of frequent serotypes identified from chicken carcasses retailers globally, even though Salmonella serotypes differ widely (Li et al., Abd-Elghany al.. 2015). 2020. et 60-90% of Additionally, human salmonellosis globally is associated with S.

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Typhimurium, followed by S. Enteritidis (Thung et al., 2018). As a result, their increased incidence among Salmonella chicken isolates found in carcasses purchased at the grocery store level is taken into consideration as a potential food safety concern. Amongst the extremely common food-borne infections in the Middle East and North Africa is salmonellosis, which places a heavy financial burden on global healthcare systems (Faour-Klingbeil et al., 2020). In the USA, the cost of foodborne salmonellosis linked to chicken eating typically ranges from 1.1 to 2.8 billion annually dollars (Scharff, 2020). Salmonellosis is a contagious food poisoning infection that creates a significant socioeconomic impact on the public healthcare systems in Egypt. Despite this, Egypt lacks a global surveillance program that would provide accurate statistics on the prevalence of the disease.

The numerous virulence genes found in Salmonella serovars, which are grouped under five Salmonella pathogenicity islands, have the potential to cause serious illnesses (SPI-1 to SPI-5). The type-III secretory system (T3SS), which is composed of the proteins expressed by the SPI-1 and SPI-2 genes, is included in the penetration and adhesion of Salmonella to cells in the body (Hussain et al., 2021). A special marker called Salmonella invasion A (invA) facilitates the inclusion of pathogen cells into the target intestinal epithelium of patients (Lan et al., 2018). The Salmonella stn gene codes for an enterotoxin called stn and is linked to infection with Salmonella Typhimurium, serotypes Typhi, and Enteritidis which cause severe symptoms such as diarrhea (Prager et al., 1995).

Chicken meat is characterized by low fat and cholesterol level and lacks any cultural or religious limitations; chicken is a wellknown, affordable and healthy source of animal protein. In Egypt consumers, regardless of their level of income, prefer poultry meat, especially chicken meat. Approximately, 1.2 billion chickens or 1125 million tons of chicken flesh are consumed per year (Shatokhin et al., 2017). Chicken carcasses are contaminated mostly different processing phases, throughout including the slaughter of animals, evisceration, scalding, washing, plucking, chilling, and storage at retail stores since in small-scale processing several plants. chicken flesh has been connected to numerous human salmonellosis infections (Tarabees et al., 2017).

The procedure of gutting causes the carcasses to contain significant quantities of bacteria from feathers of birds, paws, and feces in addition to cross-contamination from the preparation area and workers (Thomas et al., 1980). Then, they are washed internally and externally using pressurized water to achieve three goals: get rid of any blood, debris, and feather residue the skin; minimize surface from contamination, and prevent further drying in the refrigeration systems. Washing is a crucial step since it gets rid of a lot of bacteria from processed carcasses, so the slaughterhouse's "clean zone" officially starts at the washing step. Moreover, operations are done in this area at carefully regulated low temperatures to prevent contamination. Therefore, water is among the most essential resources used in poultry processing plants because it is used in washing and rinsing chickens (Cogan et al., 2002). In poultry slaughterhouses, the water used for washing and rinsing the chickens must have antibacterial, such as chlorine and organic acids, moreover, renovation of the used water is necessary (Keener et al., 2004). Concerning small-scale the processing facilities, all the previous conditions are not applied, and therefore the washing water will be a source of contamination for the chicken carcasses. Consequently, washing water in different small-scale processing plants is the main reason for the cross-contamination of different types of bacteria mainly the *Enterobacteriaceae* group such as *Salmonella* and *Escherichia coli*.

Chickens raised for commercial purposes use a lot of feed. Numerous vital digestive, metabolic, and excretory processes that the liver performs have a key influence on the health and productivity of chickens (Procura et al., 2019). Chicken liver is a food item that can be utilized in a variety of dishes, including rice, soups, and sauces due to it high zinc. are iron, and vitamin content. Giblets mostly the liver may include germs, such as Salmonella, which creates a major danger to the world's global health, causes major morbidity, and has a sizable financial impact (Abd El-Aziz, 2013).

Because of the extensive overuse and abuse of antimicrobials in chicken and animal multidrug-resistant rearing. (MDR) Salmonella enterica serotypes are increasingly becoming found in food derived from animals, posing a significant risk to animal and people's health and both potentially reducing the number of effective therapeutic choices available for the prevention and treatment of various human salmonellosis (Elshebrawy et al., 2022). Salmonella isolates resistant to fluoroquinolones and cephalosporins, which have been regarded as the two greatest clinically significant antimicrobials to deal with human Salmonella poisoning, have been a large number of scientists around the world have observed over the past decades, leading to a significant increase in deaths and morbidity cases (Qiao et al., 2017).

Consequently, the goal of this research is to observe the occurrence and antimicrobial resistance of *Salmonellae* isolated from broiler's liver and washing water obtained from different small-scale poultry processing facilities.

MATERIALS AND METHODS

A total of forty-five samples from the liver of broiler chicken and ten washing water samples were collected from several shops and small-scale poultry processing plants distributed in Cairo and Giza governorate, Egypt, from September 2021 to December 2021. Each collected sample was separately packaged in a plastic bag then identified and transferred in an icebox to the Lab of Food Hygiene and Control Department, Cairo University, Egypt. Samples were investigated immediately after arrival.

2. Salmonella isolation and identification

The International Organization for Standardization's approach (ISO 6579-1, 2017) was used to isolate and identify Salmonella as follows: Each sample was transferred from its packaging to 225 ml of buffered peptone water (Oxoid, CM0509) into a sterilized plastic bag. Subsequently, violently agitated the bag was and homogenized in a stomacher for one minute at ambient temperature. Afterward, a sterile flask was used to transfer the solution, where it was incubated at 37 °C for 18 hours. 100 ul of the pre-enriched media was inoculated into 10 ml of RV (Rappaport-Vassiliadis) broth (Oxoid CM0669). The mixture was then incubated at $42 \pm 2 \text{ °C}/24 \pm 2 \text{ h}$. The enriched broth was then streaked onto the Xylose-Lysine Desoxycholate (XLD) agar (Oxoid, CM0469) surface and the XLD agar plates were incubated at 37°C for 24 h.

Red-colored colonies either with or without black cores that appeared on XLD agar plates as suspected colonies were recognized using biochemical assays, including the urease, indole, triple sugar iron, Voges-Proskauer. citrate utilization, lysine decarboxylase, and methyl-red tests. Using the primers described in (Table 1) that address the Salmonella invasion gene (invA), all Salmonella isolates that were biochemically anticipated were submitted to serotyping and molecular identification by using the polymerase chain reaction.

3. DNA extraction of bacteria

1. Sampling

Salmonella genomic DNA was isolated using the boiling technique (Reischl *et al.*, 1994).

4. Salmonella serotyping

The Kauffmann-White system was applied for the serological characterization of *Salmonella* isolates Kauffman (1974) that had undergone molecular confirmation employing commercial O and H antisera that are monovalent and polyvalent, in a reference laboratory for veterinary quality control on poultry production, Animal Health Research Institute, Dokki, Giza.

5. Molecular confirmation of Salmonella isolate

Invasion A gene (*invA*) of *Salmonella* was molecularly detected using a conventional PCR assay. The *invA* gene was amplified by using PCR. The process was carried out with a thermal pattern as follows: Denaturation (94 °C for 30 s), annealing (64 °C for 30 s), and extension (72 °C for 45 s), followed by a final extension at 72 °C for 10 min, while a particular band was found after the electrophoresis procedure at 284 bp (Rahn *et al.*, 1992).

6. Antimicrobial sensitivity testing of the isolated Salmonella

According to the procedure mentioned by the Clinical Laboratory Standards Institute's protocol (CLSI, 2020), the antimicrobial resistance profiles of each serotyped verified Salmonella serovar were assessed using the Kirby-Bauer disc diffusion standard method on Mueller-Hinton agar (Oxoid, CM0337). antimicrobials The eighteen examined belonged to 9 antibiotics categorized: Penicillins (Ampicillin, AMP, 10µg), macrolides (Azithromycin, AZM, 15µg), Cephalosporins (Cefoxitin, FOX, 10µg; Cefpodoxime, CPD, 10µg; Cefotaxime, Cefepime, CTX, 30µg; CPM, 10µg; Ceftazidime, CAZ, 30µg; aztreonam, ATM, Ceftriaxone, 30µg), 30µg; CRO, Fluoroquinolones (Ciprofloxacin, CIP, 5µg; Nalidixic acid, NA, 30µg), Aminoglycosides (Amikacin, AK, 30µg; Gentamicin, CN, 10µg), Sulphonamides (SulphamethoxazoleTrimethoprim, SXT, 25µg), Tetracycline (Tetracycline, TE, 10µg), phenicols (Chloramphenicol), Carbapenems and (Meropenem, MEM, 10µg; Ertapenem, ETP, 10µg). Salmonella isolates were divided into categories three depending on their antimicrobial resistance patterns: extensively drug-resistant (XDR), multidrug-resistant (MDR), and pan-drug-resistant (PDR). MDR Salmonella isolates exhibited resistance to at least one antibiotic agent in three or more antimicrobial classes. While they were susceptible to only one or two antimicrobial classes, they were classified as extensively drug-resistant (XDR) when they showed resistance to at least one agent in all but two or fewer antimicrobial categories, and pandrug-resistant (PDR) when they showed resistance to all drugs in all antimicrobial classes tested (Magiorakos et al., 2012).

RESULTS

1. Occurrence of Salmonella spp. in washing water and livers of broilers

Salmonella spp. in both livers and washing water was detected by traditional methods that included cultivation. biochemical tests, and genetic confirmation through using the *invA* gene, and utilizing multivalent antisera for flagellar (H) and somatic (O) antigens for serological recognition. The occurrence of Salmonella among examined chicken liver and washing water is illustrated in (Table 2). Salmonella was isolated from one chicken liver out of 45 examined livers (2.2%). The isolated strain was identified as Salmonella Anatum. Salmonella was not detected in any of the examined washing water samples (Figure 1).

2. Molecular verification of Salmonella isolates by identification of invA gene

By amplifying the 284 bp *Salmonella* gene fragment (*invA* gene) using PCR, one *Salmonella* isolate was molecularly identified as *Salmonella* (Figure 2).

3. Antimicrobial resistance profiles of Salmonella isolate

The antibiotic sensitivity patterns of one serotyped verified *Salmonella* serovar against a range of 18 antibiotics are reported in (Table 3). Commonly, a bacterium strain

could be considered an MDR when it displays resistance to a minimum of one antimicrobial drug in three or even more antimicrobial classes.

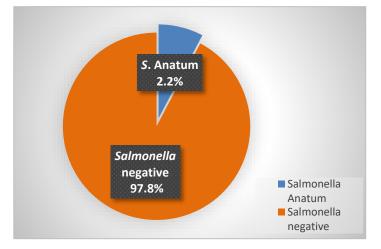


Figure 1. Occurrence of Salmonella serotype in chicken livers.

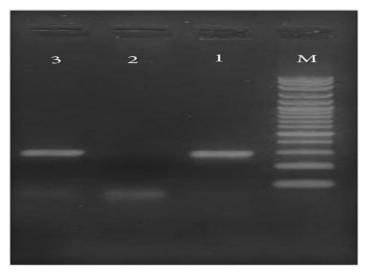


Figure 2. Occurrence of *invA* marker gene in recovered *Salmonella* Anatum from examined chicken livers.

Note: Lane M: DNA Ladder (100 bp); Lane 1: +ve control; Lane 2: -ve control; Lane 3: positive sample show specific band at 284 bp.

Target genes	Primer sequence (5'-3')	Length of PCR product	Company	References
-	F: GTGAAATTATCGCCACGTT CGGGCAA	20.41	Metabion,	(Rahn et al., 1992).
InvA	R: TCAT CGCACCGTCAAAGGAAGGAACC	284bp	Germany	

Table 1. Primer sea	uences were used fo	or the identification	of the <i>invA</i> marker gene
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Table 2. Incidence of Salmonella in	chicken liver and was	hing water
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Sample type	No. of sample	+ve sample	percentage
Liver	45	1	2.2%
Washing water	10	_	_

Table 3. Antimicrobial resistance profiles of the *Salmonella* Anatum isolate (n=1) obtained from chicken livers

Antibiotic groups	Resistance profile	AK=Amikacin;
AK	S	CN=Gentamicin;
CN	R	AMP=Ampicillin;
AMP	R	AZM=Azithromycin;
AZM	S	CIP=Ciprofloxacin;
CIP	R	NA=Nalidixic acid;
NA	S	SXT=Sulfamethoxazole-trimetho Prim;
SXT	S	TE=Tetracycline;
TE	R	C=Chloramphenicol;
С	R	FOX=Cefoxitin;
FOX	S	CPD=Cefpodoxime;
CPD	Ι	CTX=Cefotaxime; CAZ=Ceftazidime;
СТХ	R	CRO=Ceftriaxone;
CAZ	R	CPM=Cefepime;
CRO	R	ATM=aztreonam;
СРМ	R	MEM=Meropenem;
ATM	R	ETP=Ertapenem.
MEM	S	S=Susceptible.
ЕТР	S	R=Resistant.
		I=Intermediate

DISCUSSION

The occurrence of Salmonella in poultry giblets and carcasses retailers varies by country and is influenced by many factors, including rates of Salmonella infection and contamination in slaughtered bird carcasses, slaughter and preparation, methods of potential cross-contamination at the store level, handling and storage conditions, sampling and collection techniques, and variations in Salmonella isolation methods between studies. Panzenhagen et al. (2016) summarized that the incidence rate of Salmonella in poultry carcasses was 88.2% (134/152) collected from retail shops in Cambodia. Moreover, Salmonellae could be isolated from 63.6% (302/475) of China's retail chicken flesh (Zhang et al., 2018).

However, a smaller percentage of *Salmonella* in chicken carcasses have been detected by several authors in many countries, For instance, Trinidad's rate was 8.3 % (Khan *et al.*, 2018), 18% was in purchased frozen and chilled chicken carcasses in Libya, In Iraq 11.5 % (Harb *et al.*, 2018), 23.7% in India, and 33.5 percent in China (Zhu *et al.*, 2014).

Our study revealed that *Salmonella* Anatum was isolated from the chicken's liver (Figure 1). Nearly similar results were obtained by Abd-Elghany *et al.* (2015) where *S*. Anatum was detected 4.8% from chicken liver. Slightly higher results were recorded by Al-Abadi *et al.* (2011) and Molla *et al.* (2003), who could isolate *S*. Anatum from the chicken liver in a percentage of 8% and 12.9% respectively.

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Nevertheless, our finding was lesser than that achieved by Elmonir *et al.* (2017) who summarized that the incidence rate of *Salmonella* in the chicken liver was 20%. Also, Zhao *et al.* (2017) described that *Salmonella* recovered from the chicken liver in a percentage of 32%. Furthermore, El Sayed *et al.*, (2016) found that *S.* Anatum was detected in different internal organs of broiler chickens such as the intestine (35%), liver (23%), spleen (16%), heart (14%), and yolk sac (12%).

It is worthy to mention that, S. Anatum could be detected in a percentage of 3.33% (Habashy et al., 2021), 2% (Gad et al., 2018), and 2% (Abd-Elghany et al., 2015) from chicken meat samples. In addition, a higher percentage (14.6%) was stated by Mezali et al.(2012) for poultry meat. Thai et al. (2012) found that S. Anatum was the most prevalent serotype isolated from chicken meat in north Vietnam with an incidence of 15.8%.In another research. S. Anatum was detected sporadically with a prevalence rate of 0.02% (Witkowska et al., 2018) and the such finding was lower than the obtained result in our study.

Although the water used in washing and chilling employed throughout the processing steps have a cleaning effect that reduces the bacterial loads, it can also encourage crosscontamination across carcasses (GöKsoy et al., 2004, Russell, 2008). Consequently, chicken carcasses may be contaminated either with various spoilage or pathogenic microbes. In our investigation, Salmonella spp. could not be isolated from any of the samples of washing that were examined. However, water Salmonella was detected in 0.8% of washing water at different slaughterhouses (Giombelli et al., 2015). However, Hamidi et al. (2014) detected Salmonella spp. in washing water with an incidence of 10.9%. Scalding water temperature could affect or kill many pathogens and microorganisms, which is a critical step. However, this temperature may be insufficient to kill thermophiles (Firildak et al., 2015). Our result could be attributed to the uncontrolled and elevated scalding water temperature used in small-scale poultry processing plants.

The chromosomally positioned invA gene enables the quick and accurate recognition of Every reported Salmonella serotype by PCR (Eng et al., 2015, Mubarak et al., 2022). Additionally, for the genus to invade host epithelial cells, the invA gene must be present (Fàbrega et al., 2013). Primer sequences were used for the identification of the invA marker gene listed in (Table 1). Many studies from many nations have exploited the *invA* gene as a quick, accurate, and affordable indicator for the genetic verification of Salmonella species derived from chicken origins, including, Egypt (Elshebrawy et al., 2022), Iraq (Harb et al., 2018), Turkey (Cunningham et al., 2022), and South Africa (Ramtahal et al., 2022).

Salmonella is one example of a multidrugresistant (MDR) bacterium that has emerged as a result of the extensive utilization of antibacterial drugs in both human and animal medicine for improving growth, prevention, and treatments (Harb et al., 2018, Catry et al., 2015). In our study, S. Anatum showed resistance to ten antimicrobial agents among six antimicrobial classes. Additionally, S. Anatum showed resistance to chloramphenicol and tetracycline, they are regarded as the conventional first-line medications frequently used to treat salmonellosis. This pattern of resistance is consistent with that seen in other investigations carried out in South Africa (Phosa et al., 2022), China (Li et al., 2019), and Iraq (Harb et al., 2018).

Aminoglycosides are a member of the antibacterial drugs that are most frequently used to treat various diseases in both animals and people. In the current study, *S*. Anatum exhibited resistance to gentamicin, it is regarded as a crucial drug agent for regular surveillance systems for resistance of *Salmonella* species (Garcia-Migura *et al.*, 2014). This pattern of resistance is congruent with what has been observed in other investigations (Li *et al.*, 2019) as well as in (Harb *et al.*, 2018).

S. Anatum exhibited a high resistance rate towards cefotaxime, ceftriaxone, and ceftazidime(third-generation cephalosporin), This resistance pattern is compatible with results from a related study where *Salmonella*

has been recovered and isolated from chicken carcasses in South Africa (Mokgophi et al., 2021), China (Yue et al., 2020), and Iraq (Harb et al., 2018). The misuse of antibiotics for treatments, protection, and improvement of growth in chicken farming may be the cause of the greater resistance rates for ceftazidime, cefotaxime, and ceftriaxone in this research. It is noteworthy that, the preferred medications for treating virulent non-typhoidal Salmonella poisoning in human beings, particularly in children and the elderly, are fluoroquinolones third-generation cephalosporins. and Salmonella strains have been detected in chicken carcasses that have developed a resistance to cefotaxime should raise the public health alarm (Andoh et al., 2017).

Interestingly, examined S. Anatum was resistant to ciprofloxacin (second-generation quinolones). Likewise, ciprofloxacin was ineffective against Salmonella enterica serotypes obtained from various poultry species in India (Mir et al., 2015). Also, Li et al. (2022) reported a consistent pattern of resistance to ciprofloxacin among Salmonella enterica serotype isolates obtained from whole poultry carcasses in China. In contrast, in Brazil. Salmonella enterica serotypes separated from chicken carcasses were susceptible to ciprofloxacin (Panzenhagen et al., 2016). The widescale evolution of MDR Salmonella serotypes recovered from various food of animal sources showed resistance to both the first line of conventional antibacterial well drugs as as important clinical antimicrobial agents such as extendedspectrum cephalosporins and fluoroquinolones pose a serious risk to the general health due to the possibility of their transmitted to people. To safeguard the general public health, antimicrobial drugs must be used in chicken and animal husbandry in a reasonable manner. Besides, it is necessary to set up surveillance programs to track the usage of antibiotics in developing nations to protect global health from the development of illnesses that are resistant to antibiotics.

CONCLUSION

This study revealed that S. Anatum serovar was found at a percentage of 2.2% in Cairo

and Giza markets in Egypt. However, Salmonella spp. failed to be isolated from washing water used in small-scale poultry processing abattoirs. The isolated serovar exhibits a multidrug-resistant (MDR) character aminoglycosides, third-generation to cephalosporins, and fluoroquinolones. The detected S. Anatum serovar displayed resistance to both cefotaxime and ciprofloxacin, which are regarded as the standard antibiotic agents for treating Salmonella poisoning in humans. Such results may indicate a hazard to human beings either through cross-contamination or if raw and undercooked livers are consumed. To stop the transmission of Salmonella serotypes and lower the possibility of infection in humans, our research also highlighted the necessity to implement continuous monitoring systems, and Hazard Analysis Critical Control Point (HACCP) programs at each level of the manufacturing and production process. The reasonable use of antibacterial medicines in both human and animal medicine is essential to safeguard global health against the spread of multidrug-resistant bacteria. Furthermore, greater research is needed into the antibiotic susceptibility patterns of Salmonella serotypes derived from purchased chicken carcasses and giblets in the marketplaces of Egypt.

REFERENCES

- Abd-Elghany, S., Sallam, K., Abd-Elkhalek, A. and Tamura, T. (2015): Occurrence, genetic characterization and antimicrobial resistance of Salmonella isolated from chicken meat and giblets. Epidemiology & Infection, 143(5), 997-1003
- Abd El-Aziz, D.M. (2013): Detection of Salmonella Typhimurium in retail chicken meat and chicken giblets. Asian Pacific journal of tropical biomedicine, 3(9), 678-681
- Al-Abadi, I. K. M., and Al-Mayah, A. A. S. (2011). Isolation and identification of Salmonella spp. from chicken and chicken environment in Basrah province. Afr. J. Biol. Sci, 7, 33-43.
- Andoh, L.A.; Ahmed, S.; Olsen, J.E.; Obiri-Danso, K.; Newman, M.J.; Opintan, J.A.; Barco, L. and Dalsgaard, A.

(2017): Prevalence and characterization of *Salmonella* among humans in Ghana. *Tropical medicine and health*, 45(1), 1-11

- Catry, B.; Cavaleri, M.; Baptiste, K.; Grave,
 K.; Grein, K.; Holm, A.; Jukes, H.;
 Liebana, E.; Navas, A.L. and Mackay,
 D. (2015): Use of colistin-containing
 products within the European Union and
 European Economic Area (EU/EEA):
 development of resistance in animals
 and possible impact on human and
 animal health. International journal of
 antimicrobial agents, 46(3), 297-306
- Clinical Laboratory Standards Institute (CLSI).Performance Standards for Antimicrobial Susceptibility Testing (M100, 30th Edition); 2020.
- Cogan, T.; Slader, J.; Bloomfield, S. and Humphrey, T. (2002): Achieving hygiene in the domestic kitchen: the effectiveness of commonly used cleaning procedures. Journal of Applied Microbiology, 92(5), 885-892
- Cunningham, F.; Allen, J.E.; Allen, J.; Alvarez-Jarreta, J.; Amode, M.R.; Armean, I.M.; Austine-Orimoloye, O.; Azov, A.G.; Barnes, I. and Bennett, R. (2022): Ensembl 2022. Nucleic acids research, 50(D1), D988-D995
- Elmonir, W., Hegazy, M., and Asmaa, T. (2017). Extremely drug-resistant Salmonella in broiler production chain in Egypt. Life Sci J, 14(9), 82-7.
- El Sayed, A.E.S.M.; Abdel-Azeem, M.W., Sultan, S. and Abbas, A. (2016): Detection of five major pathogenicity islands in Salmonella serovars isolated from broiler chicken.
- Elshebrawy, H.A.; Abdel-Naeem, H.H.; Mahros, M.A.; Elsayed, H.; Imre, K.; Herman, V.; Morar, A. and Sallam, K.I. (2022): Multidrug-resistant Salmonella enterica serovars isolated from frozen chicken carcasses. LWT, 113647
- Eng, S.-K.; Pusparajah, P.; AB Mutalib, N.-S.; Ser, H.-L.; Chan, K.-G. and Lee, L.-H. (2015): Salmonella: a review on pathogenesis, epidemiology and antibiotic resistance. Frontiers in Life Science, 8(3), 284-293
- Fàbrega, A. and Vila, J. (2013): Salmonella enterica serovar Typhimurium skills to

succeed in the host: virulence and regulation. *Clinical microbiology reviews*, 26(2), 308-341

- Faour-Klingbeil, D. and Cd Todd, E. (2020): Prevention and control of foodborne diseases in Middle-East North African countries: review of national control systems. International Journal of Environmental Research and Public Health, 17(1), 70
- Firildak, G.; Asan, A. and Goren, E. (2015): Chicken carcasses bacterial concentration at poultry slaughtering facilities. Asian J. Biol. Sci, 8(1), 16-29
- Gad, A.H.; Abo-Shama, U.H.; Harclerode, K.K. and Fakhr, M.K. (2018): Prevalence, serotyping, molecular typing, and antimicrobial resistance of Salmonella isolated from conventional and organic retail ground poultry. Frontiers in Microbiology, 9(2653
- Garcia-Migura, L.; Hendriksen, R.S.; Fraile, L. and Aarestrup, F.M. (2014): Antimicrobial resistance of zoonotic and commensal bacteria in Europe: the missing link between consumption and resistance in veterinary medicine. Veterinary Microbiology, 170(1-2), 1-9
- Gast, R.K. and Porter, JR, R.E. (2020): Salmonella infections. Diseases of poultry, 717-753
- Giombelli, A.; Hammerschmitt, D.; Cerutti, M.F.; Chiarini, E.; Landgraf, M.; Franco, B.D. and Destro, M.T. (2015): High pressure spray with water shows similar efficiency to trimming in controlling microorganisms on poultry carcasses. Poultry Science, 94(10), 2589-2595
- Gö Ksoy, E.O.; Kirkan, S. and Kok, F. (2004): Microbiological quality of broiler carcasses during processing in two slaughterhouses in Turkey. *Poultry science*, 83(8), 1427-1432
- Habashy, A.H.; El-Dien, W.M.S.; Hussein, M.A. and Darwish, W.S. (2021): Prevalence of Staphylococcus aureus and Salmonella Species In Retailed Chicken Meat With A Reduction Trial Using Nigella sativa and Rosemary Essential Oils. Slovenian Veterinary Research, 58(299-305

- Hamidi, A.; Irsigler, H.; Jaeger, D.; Muschaller, A. and Fries, R. (2014): Quantification of water as a potential risk factor for cross-contamination with Salmonella, Campylobacter and Listeria in a poultry abattoir. British poultry science, 55(5), 585-591
- Harb, A.; Habib, I.; Mezal, E.H.; Kareem, H.S.; Laird, T.; O'dea, M. and Abraham, S. (2018): Occurrence, antimicrobial resistance and whole-genome sequencing analysis of Salmonella isolates from chicken carcasses imported into Iraq from four different countries. International journal of food microbiology, 284(84-90)
- Hussain, S.; Ouyang, P.; Zhu, Y.; Khalique, A.; He, C.; Liang, X.; Shu, G. and Yin, L. (2021): Type 3 secretion system 1 of Salmonella Typhimurium and its inhibitors: a novel strategy to combat salmonellosis. Environmental Science and Pollution Research, 28(26), 34154-34166
- ISO 6579-1. Microbiology of food and animal feeding stuff- Horizontal method for the detection of *Salmonella*. ISO, Geneva; 2017.
- Kauffman, G. (1974): Kauffmann white scheme. J. Acta. Path. Microbiol. Sci, 61(385).
- Keener, K.; Bashor, M.; Curtis, P.; Sheldon, B. and Kathariou, S. (2004): Comprehensive review of Campylobacter and poultry processing. Comprehensive reviews in food science and food safety, 3(2), 105-116
- Khan, A.S.; Georges, K.; Rahaman, S.; Abdela, W. and Adesiyun, A.A. (2018): Prevalence and serotypes of Salmonella spp. on chickens sold at retail outlets in Trinidad. PLoS One, 13(8), e0202108
- Lan, T.T.; Gaucher, M.-L.; Nhan, N.T.; Letellier, A. and Quessy, S. (2018): Distribution of virulence genes among Salmonella serotypes isolated from pigs in Southern Vietnam. Journal of food protection, 81(9), 1459-1466
- LI, Y.; Kang, X.; Ed-Dra, A.; Zhou, X.; Jia, C.; Müller, A.; Liu, Y.; kehrenberg, C. and Yue, M. (2022): Genome-Based Assessment of Antimicrobial Resistance

and Virulence Potential of Isolates of Non-Pullorum/Gallinarum *Salmonella* Serovars Recovered from Dead Poultry in China. *Microbiology spectrum*, e00965-22

- Li, Y.; Pei, X.; Zhang, X.; Wu, L.; Liu, Y.; Zhou, H.; MA, G.; Chen, Q.; Liang, H. and Yang, D. (2019):A surveillance of microbiological contamination on raw poultry meat at retail markets in China. Food Control, 104(99-104
- Li, Y., Yang, Q., Cao, C., Cui, S., Wu, Y., Yang, H., Xiao, Y. and Yang, B. (2020): Prevalence and characteristics of Salmonella isolates recovered from retail raw chickens in Shaanxi Province, China. Poultry science, 99(11), 6031-6044
- Magiorakos, A.-P.; Srinivasan, A.; Carey, R.; Carmeli, Y.; Falagas, M.; Giske, C.; Harbarth, S.; Hindler, J.; Kahlmeter, G. and Olsson-Liljequist, B. (2012): Multidrug-resistant, extensively drugresistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical microbiology and infection, 18(3), 268-281
- Majowicz, S. E., Musto, J., Scallan, E., Angulo, F. J., Kirk, M., O'Brien, S. J., and International Collaboration on Enteric Disease "Burden of Illness" Studies. (2010). The global burden of nontyphoidal Salmonella gastroenteritis. Clinical infectious diseases, 50(6), 882-889.
- Mezali, L. and Hamdi, T. M. 2012. Prevalence and antimicrobial resistance of Salmonella isolated from meat and meat products in Algiers (Algeria). Foodborne pathogens and disease, 9(6), 522-529
- Mir, I.A., Kashyap, S.K. and Maherchandani, S. (2015): Isolation, serotype diversity and antibiogram of Salmonella enterica isolated from different species of poultry in India. Asian Pacific Journal of Tropical Biomedicine, 5(7), 561-567
- Mokgophi, T.M.; Gcebe, N.; Fasina, F. and Adesiyun, A.A. (2021): Antimicrobial resistance profiles of Salmonella isolates on chickens processed and retailed at outlets of the informal market in

Gauteng Province, South Africa. *Pathogens*, 10(3), 273

- Mubarak, A., Mustafa, M., Abdel-Azeem, M. and Ali, D. 2022. Virulence and antibiotic resistance profiles of *Salmonella* isolated from chicken-ready meals and humans in Egypt. Adv. Anim. Vet. Sci, 10(2), 377-388
- Molla, B., Alemayehu, D., and Salah, W. (2003). Sources and distribution of Salmonella serotypes isolated from food animals, slaughterhouse personnel and retail meat products in Ethiopia: 1997-2002. Ethiopian Journal of Health Development, 17(1), 63-70.
- Panzenhagen, P.H.N.; Aguiar, W.S.; Da Silva Frasão, B.; De Almeida Pereira, V.L.; Da Costa Abreu, D.L.; Dos Prazeres Rodrigues, D.; Do Nascimento, E.R. and De Aquino, M.H.C. (2016): Prevalence and fluoroquinolones resistance of Campylobacter and Salmonella isolates from poultry carcasses in Rio de Janeiro, Brazil. Food Control, 61(243-247
- Phosa, M.; Fasina, F.; Morar-Leather, D. and Adesiyun, A. (2022): Prevalence and characterization of Campylobacter species from chickens sold at informal chicken markets in Gauteng, South Africa. Journal of Food Protection,
- Prager, R.; Fruth, A. and Tschäpe, H. (1995): Salmonella enterotoxin (stn) gene is prevalent among strains of Salmonella enterica, but not among Salmonella bongori and other Enterobacteriaceae. FEMS Immunology & Medical Microbiology, 12(1), 47-50
- Procura, F.; Bueno, D.J.; Bruno, S.B. and Rogé, A.D. (2019): Prevalence, antimicrobial resistance profile and comparison of methods for the isolation of Salmonella in chicken liver from Argentina. Food Research International, 119(541-546
- Qiao, J.; Zhang, Q.; Alali, W.Q.; Wang, J.; Meng, L.; Xiao, Y.; Yang, H.; Chen, S.; Cui, S. and Yang, B. (2017): Characterization of extended-spectrum β-lactamases (ESBLs)-producing Salmonella in retail raw chicken carcasses. International journal of food microbiology, 248(72-81).

- Rahn, K.; De Grandis, S.; Clarke, R.; Mcewen, S.; Galan, J.; Ginocchio, C.; Curtiss Iii, R. and Gyles, C. (1992): Amplification of an invA gene sequence of Salmonella Typhimurium by polymerase chain reaction as a specific method of detection of Salmonella. Molecular and cellular probes, 6(4), 271-279
- Ramtahal, M.A.; Somboro, A.M.; Amoako, D.G.; Abia, A.L.; Perrett, K.; Bester, L.A. and Essack, S.Y. (2022): Molecular epidemiology of Salmonella enterica in poultry in South Africa using the farmto-fork approach. International journal of microbiology, 2022.
- Reischl, U., Pulz, M., Ehret, W. and Wolf, H.J., 1994. PCR-based detection of mycobacteria in sputum samples using a simple and reliable DNA extraction protocol. *BioTechniques*, 17(5), pp.844-845.
- Russell, S. (2008): The effect of an acidic, copper sulfate-based commercial sanitizer on indicator, pathogenic, and spoilage bacteria associated with broiler chicken carcasses when applied at various intervention points during poultry processing. *Poultry Science*, 87(7), 1435-1440
- Scharff, R.L. (2020): Food attribution and economic cost estimates for meat-and poultry-related illnesses. Journal of food protection, 83(6), 959-967
- Shatokhin, Y.; El Gammal, M. and Prikhodko, D. (2017): Broiler Poultry Industry: Investment Challenges and Opportunities. Arab Republic of Egypt. Food and Agriculture Organization of the United Nations, Rome, Italy,
- Tarabees, R.; Elsayed, M.S.; Shawish, R.; Basiouni, S. and Shehata, A.A. (2017): Isolation and characterization of Salmonella Enteritidis and Salmonella Typhimurium from chicken meat in Egypt. The journal of infection in developing countries, 11(04), 314-319
- Thai, T.H.; Hirai, T.; Lan, N.T. and Yamaguchi, R. (2012): Antibiotic resistance profiles of Salmonella serovars isolated from retail pork and chicken meat in North Vietnam.

International journal of food microbiology, 156(2), 147-151

- Thomas, C. and Mcmeekin, T. (1980): Contamination of broiler carcass skin during commercial processing procedures: an electron microscopic study. Applied and Environmental Microbiology, 40(1), 133-144
- Thung, T.Y.; Radu, S.; Mahyudin, N.A.; Rukayadi, Y.; Zakaria, Z.; Mazlan, N.; Tan, B.H.; Lee, E.; Yeoh, S.L. and Chin, Y.Z. (2018): Prevalence, virulence genes and antimicrobial resistance profiles of Salmonella serovars from retail beef in Selangor, Malaysia. Frontiers in microbiology, 8(2697)
- Witkowska, D.; Kuncewicz, M.; Zebrowska, J. P.; Sobczak, J. and Sowinska, J. (2018): Prevalence of Salmonella spp. in broiler chicken flocks in northern Poland in 2014–2016. Annals of Agricultural and Environmental Medicine, 25(4)
- Yue, M., Li, X., Liu, D. and Hu, X., (2020). Serotypes, antibiotic resistance, and virulence genes of Salmonella in

children with diarrhea. *Journal of Clinical Laboratory Analysis*, *34*(12), p.e23525.

- Zhang, L.; Fu, Y.; Xiong, Z.; Ma, Y.; Wei, Y.; Qu, X.; Zhang, H.; Zhang, J. and Liao, M. (2018): Highly prevalent multidrugresistant Salmonella from chicken and pork meat at retail markets in Guangdong, China. Frontiers in microbiology, 9(2104)
- Zhu, J.; Wang, Y.; Song, X.; Cui, S.; Xu, H.; Yang, B.; Huang, J.; Liu, G.; Chen, Q. and Zhou, G. (2014): Prevalence and quantification of Salmonella contamination in raw chicken carcasses at the retail in China. Food Control, 44(198-202
- Zhao, X., Yang, J., Zhang, B., Sun, S., and Chang, W. (2017). Characterization of integrons and resistance genes in *Salmonella* isolates from farm animals in Shandong province, China. *Frontiers in microbiology*, 8, 1300.

مدي التواجد والمقاومة للمضادات الميكروبية للسالمونيلا المعزولة من كبد الدجاج وماء الغسيل المستخدم في مجازر الدواجن صغيرة الحجم

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هدفت هذه الدراسة إلى تحديد تواجد وتوصيف السالمونيلا غير التيفودية في كبد الدجاج والماء المستخدم في غسيل ذبائح الدجاج. وفي هذا الصدد تم جمع ٥٥ عينة شملت (٤٥ كبد دجاج ، و ١٠ ماء غسيل) من عدة متاجر ومصانع تجهيز الدواجن الصغيرة الحجم المتواجدة في القاهرة والجيزة بجمهورية مصر العربية. حيث أظهرت النتائج تواجد السالمونيلا اناتم بنسبة ٢,٢٪ (٤٥١) في كبد الدجاج، ولم يتم عزل السالمونيلا من ماء الغسيل. وكانت السلالة المعزولة هي سالمونيلا اناتم ومن خلال التحديد والتعريف الجزيئي لجينات الضراوة للسالمونيلا وجد انها تضمنت جين الضراوة الكروموسومية InvA ومن خلال التحديد والتعريف الجزيئي لجينات الضراوة السالمونيلا وجد انها تضمنت جين الضراوة مع سالمونيلا اناتم ومن خلال التحديد والتعريف الجزيئي لجينات الضراوة السالمونيلا وجد انها تضمنت جين الضراوة معرولة السالمونيلا اناتم ومن خلال التحديد والتعريف المريبية مقاومة السالمونيلا للمضادات الحيوية. كذلك تم فحص مقاومة الكروموسومية InvA والذي قد يلعب دورًا مهمًا في آلية مقاومة السالمونيلا للمضادات الحيوية. كذلك تم فحص مقاومة معزولة السالمونيلا اناتم لأهم المضادات الميكروبية المستخدمة في العلاج البشري وصناعة الدواجن وذلك باستخدام اختبار الحساسية، وقد أظهرت النتائج أن لديها مقاومة لجميع المضادات الميكروبية الميكسين، وبذلك باستخدام أزيثروميسين، حمض ناليديكسيك، سلفاميثوكسازول، تريميثوبريم، سيفوكسيتين، ميروبينيم، إرتابينيم، وكوليستين. وبذلك توضح الدراسة دور اكباد الدجاج في التلوث وانتقال السالمونيلا المقاومة لمضادات الميكروبات إلى السلسلة الغذائية توضح الدراسة دور اكباد الدجاج في التلوث وانتقال السالمونيلا المقاومة لمضادات الميكروبات إلى السلسلة الغذائية