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Antibiotic Resistance Pattern and Distribution of Resistance Genes in *Salmonella* Isolated from Chicken and Duck Eggs in Chhattisgarh, India

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ABSTRACT

Background: *Salmonella* is recognized as the most prevalent bacterial cause of foodborne diseases worldwide and animal-sourced foods have been reported as a common source of *Salmonella* infections among humans.

Methods: The commercial chicken eggs, backyard chicken eggs and duck eggs samples, 60 each, were processed for isolation and identification of *Salmonella.* All *Salmonella* isolates were further tested for resistance against six different antibiotics. The prevalence of virulence and antimicrobial resistance genes in the *Salmonella* isolates was determined by PCR.

Result: A total of 28 *Salmonella* isolates were recovered with an overall prevalence of 15.6% and out of them, 11.1% and 4.4% were from eggshell and egg content, respectively. All the isolates were found sensitive to Gentamicin however maximum resistance was observed against Cefotaxime. PCR results revealed that 100% of the isolates were carrying the *inv*A gene however *stn* gene was detected in 78.6% of isolates. Among presumptively identified β-lactam-resistant *Salmonella* isolates, 100% and 50% isolates harbored bla_{TEM} and bla_{CTX-M} genes, respectively whereas none of the isolates contained the bla_{SHV} gene. All tetracycline-resistant isolates harbored the *tet*A gene whereas none of the isolates carried the *tet*B gene. 100% of fluoroquinolone-resistant isolates were carrying the *gyr*A gene however *par*C gene was present only in 60% of isolates. These results indicate that drug-resistant *Salmonella* spp. were prevalent in eggs sold in the study area which can pose a serious public health problem.

Key words: Antimicrobial resistance, Eggs, Poultry, Resistance genes, *Salmonella*, Virulence genes.

INTRODUCTION

Globally foodborne diseases caused 600 million foodborne diseases and 420,000 deaths in the year 2010 and the most common causes identified were bacteria such as *Campylobacter* spp., *Salmonella enterica* and *Salmonella typhi* (WHO, 2015; Omari *et al*., 2018). Foodborne salmonellosis with nontyphoidal salmonellae is among the most prevalent causes of gastrointestinal infections worldwide. It has been estimated to cause approximately 153 million cases of gastroenteritis and 57,000 deaths (Cardoso *et al*., 2021) and is considered as the largest burden of an enteric disease which leads to 4.07 million DALYs (Disability Adjusted Life Years) (Kirk *et al*., 2015).

In humans, salmonellosis is usually associated with the consumption of contaminated foods of animal origin especially poultry products and raw or undercooked eggs (Andino and Hanning, 2015; Karimiazar *et al*., 2019). *Salmonella* spp. is the most commonly reported bacteria associated with foodborne outbreaks caused by egg and egg products (Choi *et al*., 2015). In the European Union (EU), 45.6% of the salmonellosis outbreaks were reported to be associated with the consumption of eggs and egg products in the year 2018 (European Food Safety Authority (EFSA) and European Centre for Disease Prevention and Control (ECDC), 2019). Eggs may be contaminated with *Salmonella* spp. by two possible routes; vertical and horizontal transmission. In the

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vertical or transovarian route, the egg content is directly contaminated as a result of *Salmonella* infection of the reproductive organs, before the eggs are covered by the shell components. During horizontal transmission, the shell of eggs is contaminated with feces and environmental vectors and the bacterium penetrates through the eggshell (Zubair *et al*., 2017; Cardoso *et al*., 2021).

In recent times, *Salmonella* spp. has gained substantial attention because of its pathogenic potential and ability to harbor resistance. *Salmonella* spp. pathogenicity has been related to several virulence genes which help the pathogen in adhesion and invasion mechanisms inside the host. Most of these virulence genes are located on a virulence-associated plasmid (pSTV) and region of the bacterial chromosome known as chromosomal *Salmonella* Pathogenicity Islands (SPIs). Some genes such as *inv*A are known to be involved in the adhesion and invasion of *Salmonella* into the host cell; whereas other *stn* genes are involved in the actual manifestation of pathogenic processes (Naik *et al*., 2015a). In addition, amplification of the *inv*A gene of *Salmonella* has been reported as a suitable target for PCR amplification, with potential diagnostic applications and its demonstration in *Salmonella* isolates can be epidemiologically relevant (Malorny *et al*., 2003; Favier *et al*., 2013). Among all antibiotics, β-Lactams, cephalosporins and fluoroquinolones are the most commonly used antibiotics in the poultry industry (Fardsanei *et al*., 2017). Injudicious use of antibiotics in poultry has resulted in the emergence of antibiotic-resistant strains of *Salmonella*, which leads to increased healthcare costs and clinical treatment failure (Cui *et al*., 2016).

The Indian Government has taken various steps to promote eggs as a good source of protein and the consumption of eggs have been increased considerably (Sangeetha *et al*., 2019). Different states of India are also providing free eggs to school children under the mid-day meal scheme therefore it is imperative to assess the quality of eggs sold in the local markets. The present study was conducted to assess the prevalence of *Salmonella* in poultry eggs sold in local markets in Chhattisgarh, India and to investigate the presence of some selected antimicrobial resistance genes in the drug-resistant *Salmonella* isolates.

MATERIALS AND METHODS

Sample collection

A total of 180 fresh eggs (60 backyard chicken eggs, 60 commercial/industrial chicken eggs and 60 duck eggs) samples were collected from retail markets of the Durg, Kanker and Dhamtari districts of Chhattisgarh, India between August 2020 and July 2021. Egg samples were collected in sterile plastic bags or cardboard boxes and transported immediately to the department of Veterinary Public Health and Epidemiology, College of Veterinary Science and Animal Husbandry, DSVCKV, Durg, Chhattisgarh, India. Each egg sample was analyzed for the presence of *Salmonella* on the eggshell and in the egg's internal contents.

Isolation and identification of *Salmonella* spp.

Isolation and identification of *Salmonella* spp. from egg samples was carried out according to the method described by Khan *et al*. (2021) with some modifications. For investigation of *Salmonella* contamination over the egg surface, the entire surface of the egg shell was swabbed with sterile cotton swabs soaked in buffered peptone water (BPW) (HiMedia, India). The swab was incubated in BPW at 37°C for 24 hr. For egg content contamination, eggs were sterilized by immersion in 70% alcohol for 2 min, cracked with a knife and the content was collected and homogenized. Thereafter, 25 ml of content was added to 225 ml of BPW and incubated at 37°C for 24 hr for pre enrichment. Then 1 ml of the culture was transferred to 9 ml of Tetrathionate broth (HiMedia, India) and incubated at 37°C for 24 hr for selective enrichment. The culture was streaked onto Bismuth Sulphite Agar (BSA) and Brilliant Green Agar (BGA) (HiMedia, India) and incubated at 37°C for 24-48 hr. All *Salmonella* isolates were subjected to various biochemical tests *viz*., triple sugar iron (TSI) agar, indole, methyl red (MR), Voges-Proskauer (VP), urease and citrate utilization.

In addition to conventional methods, *Salmonella* isolates were confirmed by the genus-specific polymerase chain reaction (PCR) method described by Rahn *et al*. (1992) for the detection of *inv*A gene (Table 1). Template DNA of *Salmonella* isolates for PCR was prepared by the boiling and snap chill method (Khan *et al*., 2021). *Salmonella* genomic DNA (MBT 103, MolBio™, HiMedia, India) and *Escherichia coli* isolate maintained in the departmental laboratory were used as positive and negative controls, respectively.

Antibiotic susceptibility testing (AST) and presumptive detection of ESBL producers

AST of all *Salmonella* isolates was conducted on Mueller-Hinton agar (MHA) (HiMedia, India) following the disc diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2015). The antibiotics used were Ampicillin (10 µg), Cefotaxime (30 µg), Cephalexin (30 µg), Ciprofloxacin (5 µg), Gentamicin (10 µg) and Oxytetracycline (30 µg) (HiMedia, India). Complete inhibition zone diameter was measured and results of the AST were interpreted as resistant, intermediate and sensitive as per CLSI guidelines (CLSI, 2015). *Salmonella* isolate displayed resistance to more than two different classes of antimicrobials was considered as 'Multiple Durg Resistant (MDR)' (Weill *et al*., 2006). Furthermore, the *Salmonella* isolates found resistant to Cefotaxime disc and sensitive to Cefotaxime/Clavulanic acid (30/10 ìg) discs with more than 5 mm diameter of zone of inhibition were considered as presumptive extended spectrum β-lactamases (ESBL) producers (CLSI, 2015).

Molecular detection of virulence and antibiotic resistance genes

All *Salmonella* isolates were tested for the virulence associated *stn* gene following the protocol given by Murugkar

et al. (2003) (Table 1). *Salmonella* isolates, which were identified as presumptive ESBL-producers and those that showed resistance to each category of antibiotics, were examined for the presence of resistance genes. *Salmonella* isolates phenotypically identified as ESBL-producers were tested for bla_{CTX-M}, bla_{TEM} and bla_{SHV} genes and those displaying resistance to tetracycline and fluoroquinolones were tested for *tet*A, *tet*B and *gyr*A, *par*C genes, respectively (Table 1). PCR amplification was performed using Veriti® 96-Well Thermal Cycler (Applied Biosystems, Singapore). Amplified PCR products were analyzed through electrophoresis on ethidium bromide stained 1.5% (w/v) agarose gel and recorded using a Gel Documentation System (Gel Doc™ XR, Biorad, USA).

RESULTS AND DISCUSSION

Prevalence of *Salmonella* in eggs

Out of 180 egg samples tested, a total of 28 *Salmonella* isolates were recovered (Table 2). All the isolates were initially identified by biochemical tests and further confirmed by detecting genus-specific *inv*A gene using PCR. The highest prevalence was observed in commercial chicken eggs (20%) followed by backyard chicken eggs (18.3%) and the least in duck eggs (8.33%). A similar prevalence rate of 20% in commercial chicken eggs was observed in a study from Tamil Nadu, India (Sangeetha *et al*., 2019). However, lower prevalence rates of 5.6%, 3.3% and 0% were reported from China (Li *et al*., 2020), Argentina (Favier *et al*., 2013) and Iran (Karimiazar *et al*., 2019), respectively. Data on the occurrence of *Salmonella* in backyard chicken eggs is very scarce. As compared to the present study (18.3%), lower prevalence rates of 10% and 1.66% were documented in backyard chicken eggs from West Bengal, India (Samanta *et al*., 2014) and Iran (Karimiazar *et al*., 2019), respectively. Moreover, the studies from Spain (Fenollar *et al*., 2019) and Egypt (Eid *et al*., 2015) have reported the absence of *Salmonella* in backyard eggs. In duck eggs, 8.33% prevalence was recorded in the present study, on the contrary, a lower prevalence rate of 1.4% was reported from England (Owen *et al*., 2016) and 0% prevalence rates were reported in studies from New York (Baker *et al*., 1985) and Malaysia (Nor Faiza *et al*., 2013). In the present study, prevalence of *Salmonella* in commercial chicken eggs was found comparatively higher than the backyard chicken eggs, it may be due to the contamination of eggs during their supply from poultry farms to wholesale and retail markets. Also poor hygiene and handling of eggs at the site of sale could be a source of contamination (Shahzad *et al*., 2012). The occurrence of *Salmonella* in backyard chicken eggs may

Table 1: Primers* used for detection of virulence and antimicrobial resistance genes.

Virulence gene or antimicrobial(s)	Target gene	Nucleotide sequence (5′-3′)	Annealing temperature (°C)	Product size (bp)	Reference
Virulence gene	*inv*A	F: GTGAAATTATCGCCACGTTCGGGCAA	55	284	Rahn *et al.* (1992)
		R: TCATCGCACCGTCAAAGGAACC			
Virulence gene	*stn*	F: TTGTGTCGCTATCACTGGCAACC	59	617	Murugkar *et al.* (2003)
		R: ATTCGTAACCCGCTCTCGTCC			
β-lactams	bla_{TEM}	F:TCGCCGCATACACTATTCTCAGAATGA	60	445	Akpaka *et al.* (2010)
		R: ACGCTCACCGGCTCCAGATTTAT			
	bla_{CTX-M}	F:ATGTGCAGYACCAGTAARGTKATGGC	60	593	Boyd *et al.* (2004)
		R:TGGGTRAARTARGTSACCAGAAYCAGCGG			
	bla_{SHV}	F: ATGCGTTATATTCGCCTGTG	60	747	Paterson *et al.* (2003)
		R: TGCTTTGTTATCGGGCCAA			
Tetracycline	*tet*A	F: GCTACATCCTGCTTGCCTTC	59	210	Titilawo *et al.* (2015)
		R: CATAGATCGCCGTGAAGAGG			
	*tet*B	F: TTGGTTAGGGGCAAGTTTTG	59	359	Titilawo *et al.* (2015)
		R: GTAATGGGCCAATAACACCG			
Fluoroquinolone	*gyr*A	F: CGTCGCGTACTTTACGCCATGAACG	52	586	Dasgupta *et al.* (2018)
		R: ATACCTTGCCGCGACCGGTACGG			
	*par*C	F: TGTATGCGATGTCTGAACTG	52	265	Dasgupta *et al.* (2018)
		R: CTCAATAGCAGCTCGGAATA			

* The specific primers used in the study were synthesized by Eurofins Genomics, India.

Table 2: Prevalence *Salmonella* in chicken and duck eggs in Chhattisgarh, India (N=180).

Source	No. of samples analyzed	No. of samples tested positive (%)	No. of samples tested positive in egg shell (%)	No. of samples tested positive in egg content (%)
Commercial chicken eggs	60	12 (20)	9 (15)	3 (5)
Backyard chicken eggs	60	11 (18.3)	9 (15)	2 (3.3)
Duck eggs	60	5 (8.3)	2 (3.3)	3 (5)

be attributed to different factors *viz.* backyard chickens access to outdoors spaces, physical contact with other farm animals and birds and absence of biosecurity, vaccination, hygiene practices *etc* (Ferreira *et al*., 2020).

In this study among 28 *Salmonella* isolates, 11.1% and 4.4% were recovered from eggshell and egg content, respectively. However, higher prevalence rates of 34.1% and 12.7% from eggshell and egg content were reported from Pakistan (Shahzad *et al*., 2012). On the contrary, lower prevalence rates of 6.1% and 1.8% were also recorded in Coimbatore, South India (Suresh *et al*., 2006). A higher prevalence of *Salmonella* was observed on the egg surface in the present study which may be due to the fact that egg surface was contaminated with feces during lay in unhygienic conditions or also from infected poultry (Paul *et al*., 2017). Contamination of eggshell possess a high risk for the consumers because it may cross contaminate the egg contents and other foodstuffs or may directly infect the consumers (Martelli and Davies, 2012). Penetration of bacteria from the egg surface into the egg content has been already demonstrated (Gole *et al*., 2014). The presence of *Salmonella* in egg contents may be due to the ability of transovarial transmission of *Salmonella* from birds to eggs (Taddese *et al*., 2019).

Table 3: Antibiogram profiles of *Salmonella* isolates (N=28) obtained from egg samples.

Antibiotic resistance pattern	Antibiotype	No. (%) of *Salmonella* isolates
O-CN-AMP-CTX	Ab1	1 (3.6)
O-AMP-CTX	Ab2	1 (3.6)
O-AMP-CIP	Ab4	1 (3.6)
CN-AMP-CTX	Ab3	2 (7.1)
O-CTX	Ab5	1 (3.6)
AMP-CTX	Ab6	3 (10.7)
CN-CTX	Ab7	2 (7.1)
CN-AMP	Ab8	1 (3.6)
CTX	Ab9	4 (14.3)
O	Ab10	3 (10.7)
AMP	Ab11	2 (7.1)
CN	Ab12	1 (3.6)
Sensitive to all of the antibiotics tested	Ab13	6 (21.4)

O; Oxytetracyclin, CN; Cefalexin, CIP; Ciprofloxacin, GEN; Gentamicin, CTX; Cefotaxime, AMP; Ampicillin.

Antibiogram profiles of *Salmonella* isolate

The results of the AST for all 28 *Salmonella* isolates are presented in Table 3 and Fig 1. All of the *Salmonella* isolates were susceptible to Gentamicin. However, a high prevalence of resistance against Cefotaxime (50%) and Ampicillin (39.3%) was observed. The degree of resistance among *Salmonella* isolates ranges from 3.57 to 50% was recorded against five antibiotics. Of all the isolates, 5 (17.9%) were identified as MDR. Furthermore, the *Salmonella* isolates also exhibited 12 different antibiotic resistance patterns (Table 3). Results further revealed that isolates recovered from egg surface showed the highest resistance against Cefotaxime (55%) and Ampicillin (40%) whereas from egg content 37.5% isolates showed resistance against Cefotaxim and Ampicillin. In the present study, the susceptibility of *Salmonella* isolates to Gentamicin is concurs with previous reports from Tamil Nadu, India (Sangeetha *et al*., 2019) and South Western Ethiopia (Taddese *et al*., 2019), wherein all isolates were

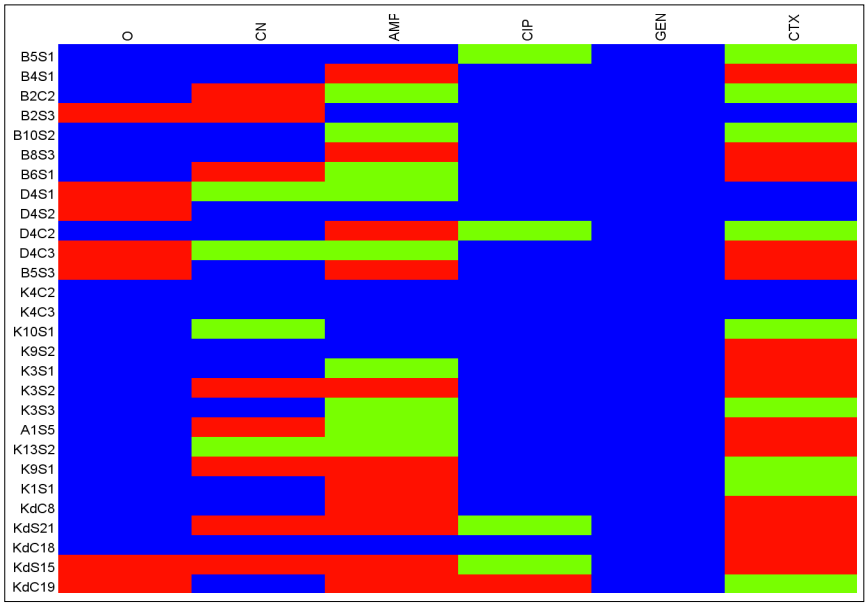

Fig 1: Heat map of antimicrobial susceptibility profiles of *Salmonella* isolates to six antibiotics as indicated by a color bar (blue = Sensitive, green = Intermediate and red = Resistant).

Table 4: Distribution of virulence and antimicrobial resistance genes among *Salmonella* isolates.

Virulence gene or antimicrobial(s)	Target genes	No. of isolates tested	Number of isolates tested positive (%)
Virulence gene	*invA*	28	28 (100)
	stn	28	22 (78.6)
β-lactams	bla_{TEM}	04¶	04 (100)
	bla_{CTX-M}	04¶	02 (50)
	bla_{SHV}	04¶	00 (0)
Tetracycline	*tet*A	07§	07 (100)
	*tet*B	07§	00 (0)
Fluoroquinolone	*gyr*A	05†	05 (100)
	*par*C	05†	03 (60)

¶ Out of 28 *Salmonella* isolates, 04 were phenotypically identified as presumptive ESBL producer and further tested for ESBL genes.
§ Out of 28 *Salmonella* isolates, 07 were found phenotypically resistant against Tetracycline and further tested for *tet*A and *tet*B genes.
† Out of 28 *Salmonella* isolates, 05 were found phenotypically resistant against Fluoroquinolone and further tested for *gyr*A and *par*C genes.

found sensitive to Gentamicin. The high level of resistance of *Salmonella* isolates against Cefotaxim and Ampicillin observed in the current study is consistent with the findings from China (Wang *et al*., 2017) and Ethiopia (Taddese *et al*., 2019). Resistance to penicillins and cephalosporins by *Salmonella* isolates is attributable to the acquired ability of the strains to produce β-lactamase enzyme. The 25% of *Salmonella* isolates showed resistance against tetracycline in this study, which is highly associated with the acquisition and expression of efflux pumps that reduce toxic levels of the drug in the bacterial cells. In *Salmonella*, these efflux pumps are mainly encoded by the *tet* genes (Hur *et al*., 2012). Strong selective pressure due to exposure to frequently used antibiotics could be one of the main reasons behind the emergence of such antibiotic-resistant *Salmonella* strains (Das *et al*., 2021). Excessive and irrational use of antibiotics with improper dosages in poultry industries either as growth promoters or for prophylactic purposes may lead to the development of MDR strains involving genetic and biochemical mechanisms. Such resistant strains have prolonged which increases their survivability and can pass to humans through the consumption of contaminated eggs (Karimiazar *et al*., 2019).

Distribution of virulence and antibiotic resistance genes in *Salmonella* isolates

In the present study, all 28 (100%) *Salmonella* isolates were harbored *inv*A virulence gene. Our results are in agreement with the similar studies from Iran (Fardsanei *et al*., 2017), Chile (Retamal *et al*., 2015) and the United States (Han *et al*., 2013), where *inv*A gene was detected in 100% *Salmonella* isolates. The *inv*A gene sequences are unique and conserved in almost all strains of *Salmonella* (Naik *et al*., 2015b; Wajid *et al*., 2019). Results further revealed that 22 (78.6%) *Salmonella* isolates harbored *stn* virulence gene, which is in line with the finding from Iran (Fardsanei *et al*., 2017). These virulence genes appear to have influence the severity of *Salmonella* infections and manifest the pathogenic process in the host cell (Fardsanei *et al*., 2017).

Out of 28 *Salmonella* isolates, 4 (14.3%) recovered from commercial chicken eggs were phenotypically identified as presumptive ESBL producer. Similarly, 14.2% and 8% isolates were found phenotypically positive for ESBL production from South India (Pradeep *et al*., 2018) and Egypt (Abdel-Maksoud *et al*., 2015), respectively. Furthermore, among four phenotypically β-lactam-resistant *Salmonella* isolates, 4 (100%) and 2 (50%) isolates harbored bla_{TEM} and bla_{CTX-M} genes, respectively whereas none of the isolates contained bla_{SHV} gene (Table 4). Our results are in agreement with the findings from China (Zhu *et al*., 2017) and Bangladesh (Parvin *et al*., 2020), where authors reported that among β-lactam-resistant *Salmonella* isolates bla_{TEM} gene was most prevalent followed by bla_{CTX-M} gene. Results further revealed that, all 7 (100%) tetracycline resistant *Salmonella* isolates contained the *tet*A gene and none of the isolates were found positive for *tet*B gene (Table 4), which is in line with the findings of some previous studies (Zamil *et al*., 2021; Das *et al*., 2021). In five fluoroquinolone resistant *Salmonella* isolates, *gyr*A and *par*C genes were detected in 100% and 60% isolates, respectively (Table 4), which is in agreement with the previous findings (Wajid *et al*., 2019).

CONCLUSION

This study provides baseline data on the occurrence of MDR *Salmonella* spp. in poultry eggs in India. Findings showed that *Salmonella* spp. was prevalent in eggs from retail shops and backyard poultry farms with an overall prevalence of 15.6%. The majority of the isolates were found resistant to the routinely used antibiotics. Furthermore, this study also provided valuable information on the circulation of different virulence and antibiotic resistance genes in *Salmonella* spp. from eggs. The apparently healthy poultry can act as a reservoir and distributor for MDR *Salmonella* spp., threatens consumer's health and can be a food safety concern for public health in the region. Therefore, the poultry sector should be provided with immediate attention by the government to maintain strict hygiene and judicious use of antibiotics. Adequate and proper cooking is recommended to kill all foodborne pathogens to reduce hazards to consumers. Along with the promotion of eggs as a complete food, consumer awareness programs/campaigns related to health risks associated with raw egg consumption may also be implemented by the national regulatory agencies.

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Conflict of Interest

Authors declare that they have no conflict of interest.

REFERENCES

Abdel-Maksoud, M., Abdel-Khalek, R., El-Gendy, A., House, B.L., Gamal, R.F., Abdelhady, H.M. (2015). Genetic characterization of multidrug-resistant *Salmonella enteric* serotypes isolated from poultry in Cairo, Egypt. African Journal of Laboratory Medicine. 4: 1-7.

Akpaka, P.E., Legall, B., Padman, J. (2010). Molecular detection and epidemiology of extended spectrum beta-lactamase genes prevalent in clinical isolates of *Klebsiella pneumonae* and *E. coli* from Trinidad and Tobago. West Indian Medical Journal. 59: 591-596.

Andino, A. and Hanning, I. (2015). *Salmonella enterica*: Survival, colonization and virulence differences among serovars. Scientific World Journal. 520179. doi: 10.1155/2015/520179.

Baker, R.C., Qureshi, R.A., Sandhu, T.S., Timoney, J.F. (1985). The frequency of Salmonellae on duck eggs. Poultry Science. 64: 646-652.

Boyd, D.A., Tyler, S., Christianson, S., McGeer, A., Muller, M.P. (2004). Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum beta-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. Antimicrobial Agents Chemotherapy. 48: 3758-3764.

Cardoso, M.J., Nicolau, A.I., Borda, D., Nielsen, L., Maia, R.L., Moretro, T., Ferreira, V., Knochel, S., Langsrud, S., Teixeira, P. (2021). *Salmonella* in eggs: From shopping to consumption-A review providing an evidence-based analysis of risk factors. Comprehensive Reviews in Food Science and Food Safety. 20: 1-26.

Choi, D., Chon, J.W., Kim, H.S., Kim, D.H., Lim, J.S., Yim, J.H., Seo, K.H. (2015). Incidence, antimicrobial resistance and molecular characteristics of nontyphoidal *Salmonella* including extended-Spectrum β-lactamase producers in retail chicken meat. Journal of Food Protection. 78: 1932-1937.

Clinical and Laboratory Standards Institute (CLSI). (2015). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fifth Informational Supplement. CLSI Document M100-S25 Wayne, PA: Clinical and Laboratory Standards Institute.

Cui, M., Xie, M., Qu, Z., Zhao, S., Wang, J., Wang, Y., He, T., Wang, H., Zuo, Z., Wu, C. (2016). Prevalence and antimicrobial resistance of *Salmonella* isolated from an integrated broiler chicken supply chain in Qingdao China. Food Control. 62: 270-276.

Das, T., Rana, E.A., Dutta, A., Bostami, M.B., Rahman, M., Deb, P., Nath, C., Barua, H., Biswas, P.K. (2021). Antimicrobial resistance profiling and burden of resistance genes in zoonotic *Salmonella* isolated from broiler chicken. Veterinary Medicine and Science. 1-8.

Dasgupta, N., Paul, D., Chanda, D.D., Chetri, S., Chakravarty, A., Bhattacharjee, A. (2018). Observation of a new pattern of mutations in *gyr*A and *par*C within *Escherichia coli* exhibiting fluoroquinolone resistance. Indian Journal of Medical Microbiology. 36: 131-135.

Eid, S., Nasef, S.A., Erfan, A.M. (2015). Multidrug resistant bacterial pathogens in eggs collected from backyard chickens. Assiut Veterinary Medical Journal. 61(144): 87-103.

European Food Safety Authority and the European Centre for Disease Prevention and Control (EFSA and ECDC). (2019). The European Union One Health 2018 Zoonoses Report. EFSA Journal. 17: 5926. 10.2903/j.efsa.2019.5926

Fardsanei, F., Dallal, M.M.S., Douraghi, M., Salehi, T.Z., Mahmoodi, M., Memariani, H., Nikkhahi, F. (2017). Genetic diversity and virulence genes of *Salmonella enterica* subspecies enterica serotype Enteritidis isolated from meats and eggs. Microbial Pathogenesis. 107: 451-456.

Favier, G.I., Estrada, C.S.L., Otero, V.L., Escudero, M.E. (2013). Prevalence, antimicrobial susceptibility and molecular characterization by PCR and pulsed field gel electrophoresis (PFGE) of *Salmonella* spp. isolated from foods of animal origin in San Luis, Argentina. Food Control. 29: 49-54.

Fenollar, A., Domenech, E., Ferrus, M.A., Jimenez-Belenguer, A. (2019). Risk Characterization of antibiotic resistance in bacteria isolated from backyard, organic and regular commercial eggs. Journal of Food Protection. 82: 422-428.

Ferreira, V., Cardoso, M. J., Magalhaes, R., Maia, R., Neagu, C., Dumitraşcu, L., Teixeira, P. (2020). Occurrence of *Salmonella* spp. in eggs from backyard chicken flocks in Portugal and Romania-Results of a preliminary study. Food Control. 113: 107180.

Gole, V.C., Chousalkar, K.K., Roberts, J.R., Sexton, M., May, D., Tan, J., Kiermeier, A. (2014). Effect of egg washing and correlation between eggshell characteristics and egg penetration by various *Salmonella* Typhimurium strains. PloS One. 9: e90987. https://doi.org/10.1371/Journal.pone.0090987.

Han, J., Gokulan, K., Barnette, D., Khare, S., Rooney, A.W., Deck, J., Nayak, R., Stefanova, R., Hart, M.E. and Foley, S.L. (2013). Evaluation of virulence and antimicrobial resistance in *Salmonella enterica* serovar Enteritidis isolates from humans and chicken- and egg-associated sources. Foodborne Pathogen and Disease. 10: 1008-1015.

Hur, J., Jawale, C., Lee, J.H. (2012). Antimicrobial resistance of *Salmonella* isolated from food animals: A review. Food Research International. 45: 819-830.

Karimiazar, F., Soltanpour, M.S., Aminzare, M., Hassanzadazar, H. (2019). Prevalence, genotyping, serotyping and antibiotic resistance of isolated *Salmonella* strains from industrial and local eggs in Iran. Journal of Food Safety. 39: e12585.

Khan, R., Shakya, S., Gade, N.E., Jain, A., Patyal, A., Ali, S.L., Chandrakar, C., Hattimare, D., Pandey, A.K. (2021). Prevalence and molecular characterization of extended-spectrum blactamases genes in *Escherichia coli* and *Salmonella* spp. isolated from fish samples in Chhattisgarh, India. Indian Journal of Fishery. 68: 105-111.

Kirk, M.D., Pires, S.M., Black, R.E., Caipo, M., Crump, J.A., Devleesschauwer, B., Dopfer, D., *et al*. (2015). World Health Organization estimates of the global and regional disease burden of 22 foodborne bacterial, protozoal and viral diseases, 2010: A data synthesis. PLOS Medicine. 12: e1001921. doi: 10.1371/journal.pmed.1001921.

Li, W., Li, H., Zheng, S., Wang, Z., Sheng, H., Shi, C., Yang, B. (2020). Prevalence, serotype, antibiotic susceptibility and genotype of *Salmonella* in eggs from poultry farms and marketplaces in Yangling, Shaanxi province, China. Frontiers in Microbiology. 11: 1482. https://doi.org/10.3389/fmicb.2020.01482.

Malorny, B., Bunge, C., Helmuth, R. (2003). Discrimination of d-tartrate-fermenting and non fermenting *Salmonella* enterica subsp. enterica isolates by genotypic and phenotypic methods. Journal of Clinical Microbiology. 41: 4292-4297.

Martelli, F. and Davies, R.H. (2012). *Salmonella* serovars isolated from table eggs: An overview. Food Research International. 45: 745-754.

Murugkar, H.V., Rahman, H., Dutta, P.K. (2003). Distribution of virulence genes in *Salmonella* serovars isolated from man and animals. Indian Journal of Medical Research. 117: 66-70.

Naik, V.K., Shakya, S., Patyal, A., Gade, N.E. (2015a). Detection of virulence genes in *Salmonella* species isolated from chevon and chicken meat. Journal of Animal Research. 5: 115-118. DOI: 10.5958/2277-940X.2015.00019.4.

Naik, V.K., Shakya, S., Patyal, A., Gade, N.E., Bhoomika. (2015b). Isolation and molecular characterization of *Salmonella* spp. from chevon and chicken meat collected from different districts of Chhattisgarh, India. Veterinary World. 8: 702-706.

Nor Faiza, S., Saleha, A.A., Jalila, A., Fauziah, N. (2013). Research note occurrence of *Campylobacte*r and *Salmonella* in ducks and duck eggs in Selangor, Malaysia. Tropical Biomedicine. 30: 155-158.

Omari, R., Frempong, G.K., Arthur, W. (2018). Public perceptions and worry about food safety hazards and risks in Ghana. Food Control. 93: 76-82.

Owen, M., Jorgensen, F., Willis, C., McLauchlin, J., Elviss, N., Aird, H., de Pinna, E. (2016). The occurrence of *Salmonella* spp. in duck eggs on sale at retail or from catering in England. Letters in Applied Microbiology. 63: 335-339.

Parvin, M.S., Hasan, M.M., Ali, M.Y., Chowdhury, E.H., Rahman, M.T., Islam, M.T. (2020). Prevalence and multidrug resistance pattern of *Salmonella* carrying extended-spectrum β-Lactamase in frozen chicken meat in Bangladesh. Journal of Food Protection. 83: 2107-2121.

Paterson, D.L., Hujer, K.M., Hujer, A.M., Yeiser, B., Bonomo, M.D. (2003). Extended spectrum â- lactamases in *Klebsiella pneumonia* blood stream isolates from seven countries: Dominance and widespread prevalence of SHV-and CTX-M-type b-lactamases. Antimicrobial Agents and Chemotherapy. 47: 3553-3560.

Paul, P., Akther, S., Ali, M.Z., Banu, H., Khan, M.S.R., Khatun, M.M. (2017). Isolation, identification and antibiogram study of *Salmonella* spp. from poultry farm environment. International Journal of Animal Biology. 3: 5-11.

Pradeep, G., Mendem, S.K., Math, G.C., Shivannavar, C.T., Gaddad, S.M. (2018). Prevalence of bla_{SHV} gene in Cephalosporin resistant *Salmonella* isolates from meat samples in south India. International Journal of Scientific Research in Biological Sciences. 5: 82-86.

Rahn, K., De Grandis, S.A., Clarke, R.C., McEwen, S.A., Galan, J.E., Ginocchio, C., Gyles, C.L. (1992). Amplification of an *inv*A gene sequence of *Salmonella* typhimurium by polymerase chain reaction as a specific method of detection of *Salmonella*. Molecular and Cellular Probes. 6: 271-279.

Retamal, P., Fresno, M., Dougnac, C., Gutierrez, S., Gornall, V., Vidal, R., Vernal, R., Pujol, M., Barreto, M., Gonzalez-Acuna, D., Abalos, P. (2015). Genetic and phenotypic evidence of the *Salmonella enterica* serotype Enteritidis human-animal interface in Chile. Frontiers in Microbiology. 6: 464. doi: 10.3389/fmicb.2015.00464.

Samanta, I., Joardar, S.N., Das, P.K., Sar, T.K., Bandyopadhyay, S., Dutta, T.K., Sarkar, U. (2014). Prevalence and antibiotic resistance profiles of *Salmonella* serotypes isolated from backyard poultry flocks in West Bengal, India. The Journal of Applied Poultry Research. 23: 536-545.

Sangeetha, A., Balakrishnan, S., Porteen, K., Dhanalakshmi, M., Manimaran, K. (2019). Prevalence and antibiotic sensitivity pattern of *Salmonella* isolated from eggs sold in commercial markets in Thanjavur, Tamil Nadu. Journal of Entomology and Zoology Studies. 7: 1026-1029.

Shahzad, A., Mahmood, M.S., Hussain, I., Siddique, F., Abbas, R.Z. (2012). Prevalence of *Salmonella* species in hen eggs and egg storing-trays collected from poultry farms and marketing outlets of Faisalabad, Pakistan. Pakistan Journal of Agricultural Sciences. 49: 565-568.

Suresh, T., Hatha, A.A.M., Sreenivasan, D., Sangeetha, N., Lashmanaperumalsamy, P. (2006). Prevalence and antimicrobial resistance of *Salmonella* enteritidis and other *salmonella* in the eggs and egg-storing trays from retails markets of Coimbatore, South India. Food Microbiology. 23: 294-299.

Taddese, D., Tolosa, T., Deresa, B., Olani, A., Shumi, E. (2019). Antibiograms and risk factors of Salmonella isolates from laying hens and eggs in Jimma Town, South Western Ethiopia. BMC Research Notes.12: 1-7.

Titilawo, Y., Obi, L., Okoh, A. (2015). Antimicrobial resistance determinants of *Escherichia coli* isolates recovered from some rivers in Osun State, South-Western Nigeria: Implications for public health. Science of the Total Environment. 523: 82-94.

Wajid, M, Awan, A.B., Saleemi, M.K., Weinreich, J., Schierack, P., Sarwar, Y., Ali, A. (2019). Multiple drug resistance and virulence profiling of *Salmonella enterica* serovars Typhimurium and Enteritidis from poultry farms of Faisalabad, Pakistan. Microbial Drug Resistance. 25: 133-142.

Wang, Y., Zhang, A., Yang, Y., Lei, C., Jiang, W., Liu, B., Wang, H. (2017). Emergence of *Salmonella enterica* serovar Indian and California isolates with concurrent resistance to cefotaxime, amikacin and ciprofloxacin from chickens in China. International Journal of Food Microbiology. 262: 23-30.

Weill, F.X., Guesnier, F., Guibert, V., Timinouni, M., Demartin, M., Polomack, L., Grimont, P.A. (2006). Multidrug resistance in *Salmonella* enteric serotype Typhimurium from humans in France (1993 to 2003). Journal of Clinical Microbiology. 44: 700-708.

World Health Organization (WHO). (2015). WHO Estimates of the Global Burden of Foodborne Diseases: Foodborne Disease Burden Epidemiology Reference Group 2007-2015. World Health Organization: Geneva, Switzerland, 2015.

Zamil, S., Ferdous, J., Zannat, M.M., Biswas, P.K., Gibson, J.S., Henning, J., Hoque, M.A., Barua, H. (2021). Isolation and antimicrobial resistance of motile *Salmonella enterica* from the poultry hatchery environment. Veterinary Research Communications. 45: 277-284.

Zhu, Y., Lai, H., Zou, L., Yin, S., Wang, C., Han, X., Liu, S. (2017). Antimicrobial resistance and resistance genes in *Salmonella* strains isolated from broiler chickens along the slaughtering process in China. International Journal of Food Microbiology. 259: 43-51.

Zubair, A.I., Al-Berfkani, M.I., Issa, A.R. (2017). Prevalence of *Salmonella* species from poultry eggs of local stores in Duhok. International Journal of Research in Medical Sciences. 5: 2468-2471.