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MOLECULAR CHARACTERIZATION OF *SALMONELLA ENTERICA SEROVARS* IN BROILER CHICKENS AT KAFR EL-SHEIKH GOVERNORATE, EGYPT

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ABSTRACT

Salmonella spp. are one of the most frequently reported foodborne pathogens worldwide. The present study investigated the virulence genotypes and antimicrobial sensitivity of *Salmonella* spp. isolated from broiler chickens in Kafr El-Sheikh governorate, Egypt. A total of 180 samples [liver (n=70), spleen (n=70) and caecum (n=40)] collected from 50 different broiler chicken flocks were used to isolate *Salmonellae*. All suspected samples were examined through a series of conventional bacteriological, biochemical, and serological techniques for isolation and identification of *Salmonella* spp. All isolates were tested for susceptibility to 16 antimicrobials. Virulence factors were determined using the polymerase chain reaction assays targeting the *inv*A, *pef*A, *avr*A, *sop*B and *spv*C. The overall isolation percentage of *Salmonella* was 6.1%. Eleven *Salmonella* isolates belonging to four different serovars were recovered. *S.* Belgdam was the most predominant species (7/11, 63.6%), followed by *S.* Typhimurium (2/11, 18.2%), and *S.* Virchow and *Salmonella* enterica subsp. Salamae (*S.* Salamae) (1/11, 9.1% each). All the isolates were positive for all tested genes except *S*. Salamae, which harbored neither the *sop*B nor *avr*A genes. All isolates exhibited resistance to almost all antimicrobials used. The finding of the present study show high positivity of virulence genes as well as multidrug resistance of all serotypes suggesting pathogenic *Salmonella* strains.

Keywords: *Salmonella*; Chicken; cPCR; Virulence genes; Antimicrobials.

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INTRODUCTION

The genus *Salmonella* comprises facultative intracellular, gram-negative, short rod-shaped, motile bacteria classified within family of *Enterobacteriaceae* (Bell, 2002; OIE, 2004). The genus has been broadly categorized into two species: *S. enterica* and *S. bongori* (CDC, 2013). To date, over 2600 different *Salmonella* serovars belonging to *S. enterica* have been reported globally, and many of these serovars are capable of causing a variety of diseases in a wide range of hosts (Mezal *et al*., 2014). These serovars are distinguished on the basis of differences in their somatic (O), flagellar (H) and capsular (K) antigens (Amagliani *et al*., 2012). Salmonellosis remains one of the most frequent bacterial diseases, affecting a wide range of poultry and causing high rates of morbidity and mortality as well as considerable economic losses to the poultry industry (Rostagno *et al*., 2006). Chickens have been involved in most *Salmonella* infections because they carry this pathogen in their guts (Black, 2008).

The ability of *Salmonella* serovars to cause systemic infections in the host is attributed to a variety of virulence genes (Murugkar *et al*., 2003). The majority of these virulence genes are grouped together in so-called *Salmonella* Pathogenicity Islands (SPIs) (Marcus *et al*., 2000; Bayoumi and Griffiths, 2010; Card *et al*., 2016). At least 60 virulence genes have been recognized within SPIs (Groisman and Ochman, 1997), with each playing a role in promoting infection by different mechanisms, including adhesion, invasion, fimbrial expression, toxin production, antibiotic resistance, systemic infection, iron acquisition and intracellular survival (Skyberg *et al*., 2006; Majowicz *et al*., 2010; Kim and ju Lee, 2017).

In *Salmonella* pathogenicity island 1, the *inv*A gene is associated with invasion of epithelial cells and delivery of the type III secretion system (TTSS) virulence associated proteins (effector proteins) into host cell (Suez *et al*., 2013; El-Sharkawy *et al*., 2017). Furthermore, because *inv*A is conserved in all *Salmonella* genus members, it has been used by many researchers as a biomarker for *Salmonella* spp. (Fekry *et al*., 2018; Mthembu *et al*., 2019). The *pef*A virulence gene, is mainly involved in intestinal adhesion as well as invasion of host cell in some *Salmonella* serotypes (Bäumler *et al*., 1997). Meanwhile, *avr*A and and *sop*B encode multiple-function effector proteins that may facilitate endothelial uptake and invasion through the TTSS enhancing the

enteritis pathway, with critical roles in suppressing inflammation and regulating epithelial apoptosis (Foley *et al*., 2008). Specifically, *avr*A encodes a TTSS effector protein that contributes to *Salmonella* virulence by suppressing the inflammatory responses of the host through the induction of cell apoptosis (particularly macrophages) in addition to suppression of IL-8 and TNF (Collier-Hyams *et al*., 2002; Ben-Barak *et al*., 2006).

Another important virulence factor for *Salmonella* is *sop*B, which plays an important role in the stimulation of secretory pathways, inducing inflammation (by attracting neutrophils to the sites of infection) and altering ion balances within cells, resulting in fluid release in the gastrointestinal tract and consequent diarrhea (Norris *et al*., 1998). Numerous *Salmonella* serovars have various enormous virulence plasmids that encode an approximately 8-Kb, highly conserved region called the *spv*C (*Salmonella* plasmid virulence) operon (Nakano *et al*., 2012). This operon contains five genes designated *spv*RABCD that are necessary for intracellular survival of *Salmonella* within host cells and rapid growth (Rotger and Casadesús, 1999). Therefore, this operon is crucial for inducing the systemic infection (Libby *et al*., 1997).

Antimicrobial resistance among *Salmonella* spp. has been emerged in the last few years, making treatment and control of infections difficult (Arkali and Çetinkaya, 2020). Indiscriminate use of antibiotics considers one of the main cause of developing multidrug resistant (MDR) bacteria including *Salmonella* serotypes (Okeke *et al*., 2005). These resistant strains pose a threat, not only to poultry, but also have the potential to infect human causing serious systemic infections (Ma *et al*., 2007). Several previous investigations have documented that different *Salmonella* serotypes such as *S*. Typhimurium, *S*. Paratyphi, *S*. Typhi, *S*. Heidelberg, *S*. Infantis, *S*. Newport, *S*. Agona and *S*. Hadar showed resistance to various antimicrobials (Mathole *et al*., 2017; Zhao *et al*., 2017; Thung *et al*., 2018). Therefore, the main objectives of this study were to determine the prevalent *Salmonella* serotypes in broiler chickens isolated from different chicken flocks in Kafr El-Sheikh governorate; and to assess the virulence genes of the isolated *Salmonellae* using conventional polymerase chain reaction (cPCR) assay. Moreover, the current study highlighted the susceptibility of *Salmonella* serotypes to different antimicrobial agents commonly used for treatment of *Salmonella* infection in poultry.

MATERIALS AND METHODS

Sampling: From April 2020 to January 2021, a total of 180 clinical samples of liver (n=70), spleen (n=70), and caecum (n=40) were collected from 50 different diseased and freshly slaughtered broiler chicken flocks (aged 3-28 days) within different localities in Kafr El-Sheikh governorate which located in the northern region of Egypt between 31° 06’ 42 North and 30° 56’ 45 East. The collected samples were labelled and immediately transferred to the laboratory in an ice bag without delay for bacteriological investigation.

Isolation, morphological and biochemical identification of *Salmonella* spp.: The standard bacteriological procedures for isolation and identification of *Salmonella* serovars were performed according to the International Organization for Standardization (ISO) 6579 (International Organization for Standardization, 2002). Approximately 2 g of each visceral organ was homogenized, pre-enriched in sterile buffered peptone water (Oxoid, UK), and incubated at 37°C for 24 hours. Then, approximately 0.l of the homogenate was aseptically inoculated into 10 mL of Rappaport Vassiliadis (RV) broth (Oxoid, UK) and incubated aerobically at 42°C for 24-48 hours. Subsequently, a loopful from each broth was subcultured onto Xylose Lysine Deoxycholate (XLD) agar (Oxoid, UK) and MacConkey agar (Oxoid, UK) plates, and incubated aerobically at 37°C for 24-48 hours. The resulting presumptive colonies showing the morphological characteristics of *Salmonella* (pink, with or without black center on XLD and pale yellow on MacConkey agar) were picked and purified on nutrient agar slants as pure cultures. Suspected *Salmonella* colonies were biochemically identified as described in ISO 6579 guidelines (International Organization for Standardization, 2002). The identities of all bacterial isolates were confirmed using a set of standard biochemical tests that included triple sugar iron agar (TSI), indole production, citrate utilization, and urease tests. The remaining pure culture of each *Salmonella* isolate was stored as a 60% glycerol stock at -80°C for further use.

Serotyping of *Salmonella* isolates: Typical *Salmonella* isolates were serotyped according to Kauffmann-White scheme (Kauffmann, 1974). This was applied through a standard slide agglutination test for the somatic and flagellar antigens using specific commercial polyvalent and monovalent *Salmonella* antisera (SINIF Co., Germany).

Antimicrobial susceptibility testing: All identified *Salmonella* isolates were tested for their susceptibility to 16 antimicrobial agents (Oxiod, Hampshire, UK) using a Kirby–Bauer disc diffusion assay according to guidelines described by the Clinical and Laboratory Standards Institute (CLSI, 2017). The following antimicrobial agents were tested: amoxicillin (AM), 10 µg; ciprofloxacin (CIP), 5 µg; cefotaxime (CTX), 30 µg; chloramphenicol (C), 30µg; streptomycin (STR), 10 µg; nalidixic acid (NAL), 30 µg; trimethoprim-sulfamethoxazole (SXT), 25 µg; gentamicin (CN), 10 µg;

tetracycline (TE), 30 µg; enrofloxacin (ENR), 5 µg; vancomycin (VA), 30 µg; kanamycin(K), 30 µg ; oxacillin (OX), 1 µg; ceftriaxone (CRO), 30 µg; erythromycin (E), 15 µg; and cefoxitin (Fox), 30 µg. Interpretation of the results of the *Salmonella* isolates was achieved using the resistant breakpoints published by CLSI, (2017).

Molecular detection of *Salmonella* virulence genes

Extraction of DNA: The stored pure cultures (0.1 mL /isolate) were cultured overnight at 37°C in tryptose soy broth (Oxoid, UK). The DNA was extracted from all isolates using the QIAamp DNA mini kit in accordance with manufacturer's instructions. Finally, the DNA was eluted with 50 µL of elution buffer and then stored at -20°C until subsequent PCR assays for the detection of target virulence genes. A positive control was prepared by extracting genomic DNA from a reference *Salmonella* strain.

Confirmation of *Salmonella* and detection of target virulence genes using cPCR: Based on publication of several investigators, five oligonucleotide primers pairs were used to detect the following *Salmonella* virulence genes: *inv*A, *pef*A, *avr*A, *sop*B, and *spv*C. The primer sequences, their corresponding target genes, and the relevant references are depicted in Table 1. First, the *inv*A gene is a biomarker for *Salmonella* spp. was used to confirm the identity of *Salmonella*, then subsequent PCR assays targeted the other genes in each *Salmonella* isolate. All PCR assays were performed in a total volume of 25 µL which included 12.5 µL Emerald Amp Max PCR master mix (Takara, Japan), 1 µL of 20 pmol of each forward and reverse primer, 6 µL of *Salmonella* DNA template, and 4.5 µL nuclease free water. The PCRs were performed as uniplex reactions using an Applied Biosystems 2720 thermal cycler (Applied Biosystems, Foster, CA, USA). The PCR cycling conditions were carried out as the following: initial denaturation at 94 ºC for 5 min followed by 35 cycles of denaturation at 94 ºC for 30 sec, annealing at 55 ºC for 30 sec (*inv*A, *pef*A), 58 ºC for 40 sec (*avr*A, *sop*B, *spv*C) and extension at 72 ºC for 45 sec followed by final extension at 72 ºC for 10 min. Finally, each amplified PCR products was electrophoresed on 1.5 % (w/v) agarose (Sigma-Aldrich, Co., St. Louis, MO, USA) prepared in 1X Tris Acetate EDTA (TAE) buffer, and then stained with 0.5 µg/mL ethidium bromide (Sigma-Aldrich, Co., St. Louis, MO, USA). The DNA ladder (100-600 bp) (Fermentas, Inc. Hanover, USA) was used as molecular size marker. The gel was then visualized and photographed under a UV transilluminator.

Table 1. Oligonucleotide primer sequences used for amplifying virulence genes of *Salmonella* serovars.

Target gene	Specificity/location	Oligonucleotide sequence (5'-3')	Amplicon size (bp)	Reference
*inv*A	*Salmonella* species/ SPI-1	F: GTGAAATTATCGCCACGTTCGGGC R: TCATCGCACCGTCAAAGGAACC	284	Oliveira *et al.*, (2003)
*pef*A	Plasmid encoded fimbriae/ Plasmid	F: TGTTTCCGGGCTTGTGCT R: CAGGGCATTTGCTGATTCTTCC	700	Murugkar *et al*., (2003)
*avr*A	SPI-1/ controls *Salmonella*-induced inflammation	F: CCTGTATTGTTGAGCGTCTGG R: AGAAGAGCTTCGTTGAATGTC C	422	Huehn *et al.*, (2010)
*sop*B	Translocated effector protein/ SPI-5	F: TCAGAAGRCGTCTAACCACTCTCA R: TACCGTCCTCATGCACACTCT	517	
*spv*C	Plasmid-encoded virulence/ Plasmid	F: ACCAGAGACATTGCCTTCC R: TTCTGATCGCCGCTATTCGR	467	

Ethical consideration: Ethical approval was provided from the Research, Publication and Ethics Committee of the Faculty of Veterinary Medicine, Kafrelsheikh University, Egypt. The committee ensures compliance with all relevant Egyptian legislations

RESULTS

Prevalence, identification and serotyping of *Salmonella* isolates: Out of 180 analyzed samples, *Salmonella* spp. were isolated in 6.1% (11/180) of the examined samples resembling flocks (Table 2). All isolates were identified by standard bacteriological and biochemical techniques, followed by confirmation by amplifying the *inv*A gene, which is genus-specific. A 284-bp DNA fragment, corresponding to the *inv*A gene, was detected in all suspected *Salmonella* isolates, irrespective of serovar or source of isolation. *Salmonella* strains were observed in 11.4% (8/70) from liver, 1.4% (1/70) from spleen, and 5% (2/40) from cecum samples. Serotyping of *Salmonella* cultures based on polyvalent and monovalent "O" and "H" *Salmonella* antisera identified the four serovars, *S*. Belgdam, *S*. Typhimurium, *S.* Virchow, and *S.* Salamae. *S*. Belgdam was the most frequently isolated serovar (7/11 isolates), accounting for

63.6% of all the *Salmonella* isolates. The next most frequently isolated serovar was *S.* Typhimurium (2/11 isolates, 18.2%), followed by *S.* Virchow (1/11 isolate, 9.2%) and *S.* Salamae (1/11 isolate, 9.2%). The *Salmonella* serovars that were isolated from liver consisted of *S*. Belgdam (5 isolates), *S*. Typhimurium, *S.* Salamae and *S.* Virchow (one isolate each). A single *S*. Typhimurium and two *S*. Belgdam isolates were found in spleen and cecum samples, respectively (Table 2).

Molecular detection of *Salmonella* virulence genes using PCR: All *Salmonella* isolates identified in this study were screened for the presence of five virulence genes using uniplex PCR assays. The oligonucleotides primers used to target each respective gene successfully amplified the expected products from DNA extracted from *Salmonella* isolates, generating amplicons of the expected sizes. Interestingly, all virulence genes (*inv*A, *pef*A, *avr*A, *sop*B, and *spv*C) were present in all recovered *Salmonella* serovars (Figures 1-5). However, the *sop*B and *avr*A genes were not detected in *S.* Salamae.

Antimicrobial susceptibility testing: The antimicrobial susceptibility testing revealed variable rates of resistance of *Salmonella* serotypes against the tested antimicrobial agents. The highest rate of resistance was observed against tetracycline, streptomycin, erythromycin, vancomycin amoxicillin, cefoxitin, oxacillin and nalidixic acid except *S.* Virchow which showed higher susceptibility against nalidixic acid and oxacillin. On the other hand, lower rate of resistance was observed against enrofloxacin, ciprofloxacin, streptomycin and gentamicin except *S.* Virchow and *S.* Salamae. With exception of *S.* Salamae, all *Salmonella* serotypes showed higher sensitivity to trimethoprim-sulfamethoxazole and chloramphenicol. All isolates exhibited intermediate resistance against ceftriaxone except *S.* Belgdam which showed high susceptibility.

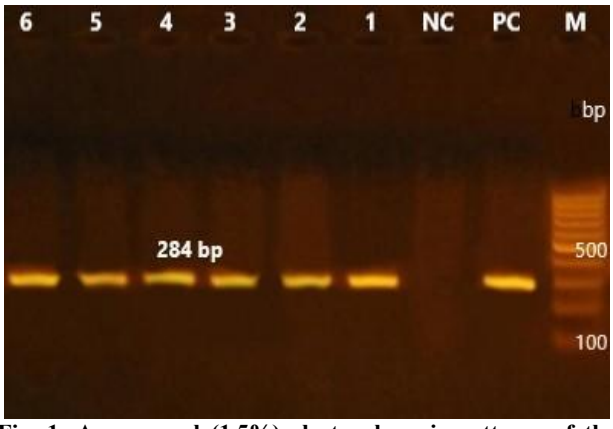

Fig. 1. Agarose gel (1.5%) electrophoresis patterns of the *inv*A PCR product. Lane M: 100 bp DNA ladder; lane PC: positive control; lane NC: negative control (deionized water); lanes (1-6): analyzed samples of *Salmonella* isolates showing positive 284 bp amplicon of *inv*A gene.

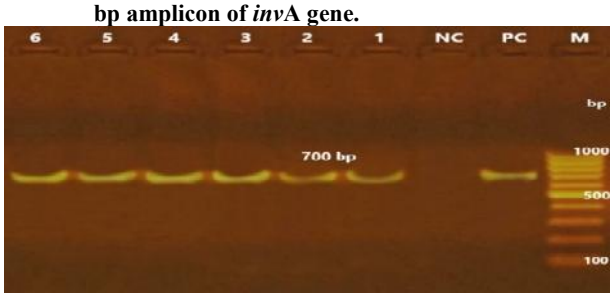

Fig. 2. Agarose gel (1.5%) electrophoresis patterns of the *pef*A gene PCR product (700 bp). Lane M: 100 bp DNA ladder; lane PC: positive control; lane NC: negative control (deionized water); lanes (1-6): positive isolates.

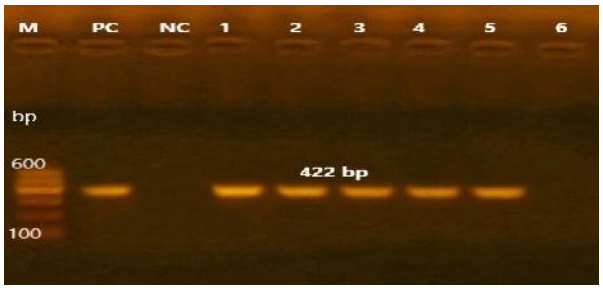

Fig. 3. Agarose gel (1.5%) electrophoresis patterns of the *avr*A gene PCR product (422 bp). Lane M: 100-600 bp DNA ladder; lane PC: positive control; lane NC: negative control; lanes (1-5): positive isolates; lane 6: negative isolate (*S.* Salamae*).*

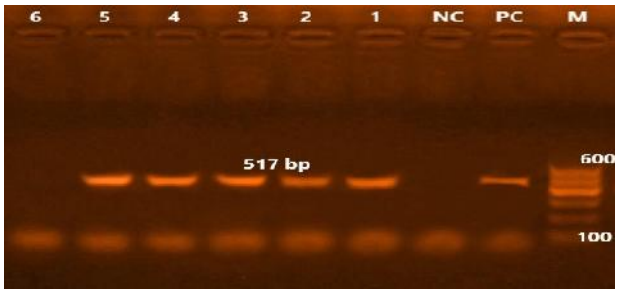

Fig. 4. Agarose gel (1.5%) electrophoresis patterns of the *sop*B gene PCR product (517 bp). Lane M: 100-600 bp DNA ladder; lane PC: positive control; lane NC: negative control (deionized water); lanes (1-5): positive isolates; lane 6: negative isolate (*S.* Salamae*).*

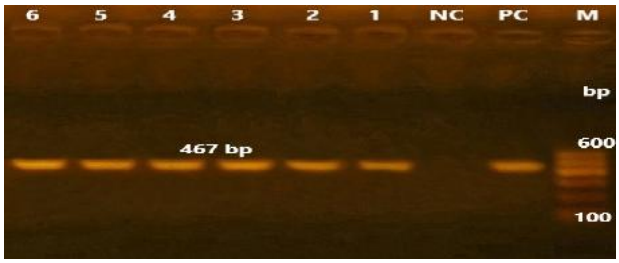

Fig. 5. Agarose gel (1.5%) electrophoresis patterns of the *spv*C gene PCR product (467 bp). Lane M: 100-600 bp DNA ladder; lane PC: positive control; lane

NC: negative control (deionized water); lanes (1- 6): positive isolates.

Table 2. Number, source, and serotyping results of *Salmonella* serovars isolated from broiler chickens.

Type of samples	No. of samples	*Salmonella* isolates		Antigenic structure profile			Serovars
		No.	%	O antigen	H antigen		
					H1	H2	
Liver	70	8	11.4	9,12	g,m,q	-	*S.* Belgdam (5 isolates)
				6.7.14	r	1.2	*S.* Virchow (1 isolate)
				1.9.12	g,m,s,t,enx	-	*S.* Salamae (1 isolate)
				1.4.5.12	i	1.2	*S.* Typhimurium (1 isolate)
Spleen	70	1	1.4	1.4.5.12	i	1.2	*S.* Typhimurium (1 isolate)
Caecum	40	2	5	9,12	g,m,q	-	*S.* Belgdam (2 isolates)

DISCUSSION

Salmonella spp. consider one of the most serious foodborne pathogens causing outbreaks and sporadic cases of human gastroenteritis worldwide (Humphrey, 2000). In poultry, *Salmonella* causes severe systemic bacterial diseases, resulting in heavy economic losses through reduced production and mortality (Haider *et al*., 2004). Chickens are infected by a wide variety of *Salmonella* serovars, with some serovars, such as *S*. Pullorum and *S*. Gallinarum, acting in a host (chicken)-specific manner, while other serovars, such as *S*. Typhimurium and *S*. Enteritidis, capable of infecting a wide range of hosts (Foley *et al*., 2008).

Previous estimates of the prevalence of *Salmonella* spp. in poultry throughout the world have varied, ranging from 4% to 92% (García *et al*., 2011; El-Sharkawy *et al*., 2017). Based on the amplification of the *inv*A gene, which is a genus specific, the overall prevalence of *Salmonella* in this study was 6.1%. This level is consistent with those previously reported in India (Samanta *et al*., 2014), Nigeria (Akeem *et al*., 2017), and Egypt (Abd El-Ghany *et al*., 2012; Tarabees *et al*., 2019). However, our findings are not consistent with results presented by Medeiros *et al*. (2011), Menghistu *et al*. (2011) and Adesiyun *et al*. (2014), who reported *Salmonella* prevalence rates of 2.7%, 2.8 % and 2.7%, respectively. Moreover, considerably higher prevalence rate have been reported in China (52.2%) (Yang *et al*., 2011), South Africa (51%) (Zishiri *et al*., 2016), India (46%) (Srinivasan *et al*., 2014), and Egypt (41%) (El-Sharkawy *et al*., 2017). Variations in the rates of isolation of *Salmonella* may be attributed to differences in the types of samples, geographic locations, *Salmonella* detection protocol used, the hygienic status of the farms, and the antibiotic regime used.

It is common for poultry to be infected by different *Salmonella* serovars. A single serovar may predominate in one country for several years, and then be replaced by another serovar in the following years (Abd El-Ghany *et al*., 2012). The dominant *Salmonella* serovar usually varies according to geographical area. However, based on recent studies, *S*. Enteritidis and *S*. Typhimurium are gaining predominance worldwide (Abd El-Ghany *et al*., 2012; Rabie *et al*., 2012). In the current study, 4 different serovars of *Salmonella* were found in all organs analyzed (liver, spleen and cecum). *S.* Belgdam was the most predominant (7 isolates), followed by *S.* Typhimurium (2 isolates). *S.* Virchow and *S.* Salamae were each represented by one isolate. These results are inconsistent with those that found *S*. Enteritidis and *S*. Typhimurium as the most frequent serotypes in Egyptian poultry farms (Abd El-Ghany *et al*., 2012; Halawa *et al*., 2016; El-Sharkawy *et al*., 2017; Elkenany *et al*., 2019). In Turkey, another recent study reported the predominance of *S*. Infantis, *S*. Enteritidis and *S*. Typhimurium in chickens (Arkali and Çetinkaya, 2020). Therefore, it is possible that new uncommon serovars have emerged, providing a possible etiology for *Salmonella* infections in Egypt.

The ability of *Salmonella* to infect a host depends mainly on presence of several virulence determinants. In the present study, all *Salmonella* serotypes (11 isolates) were screened for the presence of *inv*A, *pef*A, *avr*A *sop*B and *spv*C, which are well-recognized virulence genes belonging to different SPIs. These particular genes were selected based on their function and the potential harm they pose to poultry. Various studies from many countries have focused in investigating the prevalence of virulence genes in *Salmonella* spp. isolated from poultry (Zishiri *et al*., 2016; Khaltabadi Farahani *et al*., 2018; Elkenany *et al*.,2019; ElSheikh *et al*., 2019; Ramatla *et al*., 2019). Surprisingly, all *Salmonella* serotypes identified in the examined broiler chicken farms in this study harbor several virulence genes. Three *Salmonella* serovars harbored all examined virulence genes. The exception was *S*. Salamae, contains no *sop*B and *avr*A genes. The *inv*A gene plays an important role in invasion of the host epithelial cells during the process of *Salmonella* infection (Darwin and Miller, 1999; Salehi *et al*., 2005); and it is also regarded as a specific biomarker for *Salmonella*, because it contains sequences that are unique to various members of the genus (Ammar *et al*., 2016; Zishiri *et al*., 2016; El-Sebay *et al*., 2017). In the present study, all

Salmonella serovars harbored the *inv*A gene, which is in agreement with previous findings (Ammar *et al*., 2016; Zishiri *et al*., 2016; El-Sharkawy *et al*., 2017; Khaltabadi Farahani *et al*., 2018; Elkenany *et al*., 2019; Ramatla *et al*., 2019).

Fimbriae play an important role in *Salmonella* pathogenicity by mediating bacterial adhesion to epithelial cells. In the current study, the *pef*A gene was identified in all recovered *Salmonella* isolates, emphasizing the significance of fimbriae in the infection process. Our results agree with those of Murugkar *et al*. (2003), who reported the presence of *pef*A in 85 out of 95 *Salmonella* isolates. In contrast, other studies have not detected *pef*A gene in all *Salmonella* isolates (Muthu *et al*., 2014; Naik *et al*., 2015; Elkenany *et al*., 2019). However, our finding is higher than those reported by Hudson *et al*. (2000) and Ammar *et al*. (2016) who detected the *pef*A gene at rates of 68% and 41.18%, respectively.

The results of previous genetic analysis indicate that the *spv*C gene is necessary for the virulence phenotype of *Salmonella* (Roudier *et al*., 1992). In the current study, *spv*C was also identified in all the examined *Salmonella* isolates, which is consistent with the results of earlier studies (Castilla *et al*., 2006; Amini *et al*., 2010; Borges *et al*., 2013). Nevertheless, the results of other investigations have found that not all *Salmonella* possess this gene (Okamoto *et al*., 2009; Derakhshandeh *et al*., 2013; Moussa *et al*., 2013; Ammar *et al*., 2016; Khaltabadi Farahani *et al*., 2018).

In this study, the *sop*B and *avr*A genes were absent only in *S*. Salamae. This result is consistent with the reported *sop*B gene prevalence rate of 41.2% among *Salmonella* isolates in Egypt (Ammar *et al*., 2016). Nevertheless, other studies in Egypt (Tarabees *et al*., 2019), Iran (Khaltabadi Farahani *et al*., 2018), and India (Rahman, 2006) have found the *sop*B gene in all *Salmonella* isolates recovered from broiler chickens. Ben-Barak *et al*. (2006) have assumed that *avr*A prevalence is strongly correlated to *Salmonella* pathogenicity, and previous reports have observed this gene only in serovars that cause severe salmonellosis in humans (Hudson *et al*., 2000). In the current study, *avr*A was detected in all *Salmonella* isolates except *S*. Salamae. Another study in Egypt found this gene in all *Salmonella* isolates (ElSheikh *et al*., 2019), while in Brazil, it was found in all *S*. Enteritidis isolates (Borges *et al*., 2013). However, a lower prevalence of 30% was reported by Elkenany *et al*. (2019) in Egypt.

Antimicrobial resistance is a public health concern worldwide (Zishiri *et al*., 2016). Our findings revealed that all identified *Salmonella* strains were completely resistant to tetracycline, streptomycin, erythromycin, vancomycin amoxicillin, cefoxitin, oxacillin and nalidixic indicating that these antimicrobials have limited therapeutic values. These results were in concordance with those observed in Egypt (Khairy, 2015; Ammar *et al*., 2016), Turkey (Siriken *et al*., 2015) and South Africa (Zishiri *et al*., 2016). This high resistance may be attributed to misuse of antimicrobials either as feed additives for growth promotion or chemotherapeutic agents for treatment. In this study, the pattern of antimicrobial resistance was vary among *Salmonella* serotypes. Surprisingly, all identified *Salmonella* serotypes were resistant to at least 5 antimicrobials meaning they were MDR. Similar finding was reported in Egypt (Ammar *et al*., 2016) and Morocco (Khallaf *et al*., 2014). In this study, *S*. Virchow exhibit resistance to seven antibiotics. This result was compatible with those conducted in Nigeria, where *S*. Virchow was found to be the most resistant serotype in chickens (Fashae *et al*., 2010).

Given from the above-mentioned results, 4 *Salmonella Enterica* serovars were detected in the examined broiler chickens in Kafr El-Sheikh governorate, Egypt. Also, it can be concluded that *S.* Belgdam was the most predominant *Salmonella* serovar. Furthermore, virulence genes are widely distributed among *Salmonella* isolates regardless of source of sample, serovar, and region of sampling. The information in this report may be of value for understanding the dangerous spread of virulent serovars of *Salmonella* species raising the alarm of its zoonotic importance. The findings of this study revealed that all *Salmonella* serotypes exhibit MDR, therefore, our study support the necessity for judicious use of antimicrobial agents in veterinary medicine and regular monitoring of the antibiotic susceptibilities of isolated bacteria before treatment.

Author contributions: AAM and AMA contributed in the conception, design as well as evaluation of the study. MRS was involved in sampling, preparing for experiment and carried out the classical bacteriological and molecular techniques. IEE participated with NSR in data analysis and interpretation. IEE, NSR and MRS wrote and revised the manuscript for important intellectual content. All authors approved the manuscript for publication.

Conflict of interest: None of the authors have any conflict of interest to declare.

REFERENCES

Abd El-Ghany, W.A., S.S. El-Shafii, and M.A. Hatem (2012). survey on *Salmonella* species isolated from chicken flocks in Egypt. Asian J Anim Vet Adv. 7: 489-501.

Adesiyun, A., L. Webb, L. Musai, B. Louison, G. Joseph, A. Stewart-Johnson, S. Samlal, and S. Rodrigo (2014). Survey of *Salmonella* contamination in chicken layer farms in three Caribbean countries. J Food Prot. 77: 1471-1480.

Akeem, A.O., P. Mamman, A. Raji, C. Kwanashie, I. Raufu, and A. Aremu (2017). Distribution of virulence genes in *Salmonella* serovars isolated from poultry farm in Kwara State, Nigeria. Ceylon J. Sci. 46: 69-76.

Amagliani, G., G. Brandi, and G.F. Schiavano (2012). Incidence and role of *Salmonella* in seafood safety. Food Res. 45: 780-788.

Amini, K., T.Z. Salehi, G. Nikbakht, R. Ranjbar, J. Amini, and S.B. Ashrafganjooei (2010). Molecular detection of *inv*A and *spv* virulence genes in *Salmonella* enteritidis isolated from human and animals in Iran. Afr. J. Microbiol. Res. 4: 2202-2210.

Ammar, A.M., A.A. Mohamed, M.I. Abd El-Hamid, and M.M. El-Azzouny (2016). Virulence genotypes of clinical *Salmonella* serovars from broilers in Egypt. J. Infect. Dev. Ctries. 10: 337-346.

Arkali, A., and B. Çetinkaya (2020). Molecular identification and antibiotic resistance profiling of *Salmonella* species isolated from chickens in eastern Turkey. BMC Vet. Res. 16: 1-8.

Bäumler, A.J., A.J Gilde, R.M Tsolis, A. Van Der Velden, B. Ahmer, and F. Heffron (1997). Contribution of horizontal gene transfer and deletion events to development of distinctive patterns of fimbrial operons during evolution of *Salmonella* serotypes. J. Bacteriol. 179: 317-322.

Bayoumi, M.A., and M.W. Griffiths (2010). Probiotics down-regulate genes in *Salmonella* enterica serovar typhimurium pathogenicity islands 1 and 2. J. Food Prot. 73: 452-460.

Ben-Barak, Z., W. Streckel, S. Yaron, S. Cohen, R. Prager, and H. Tschäpe (2006). The expression of the virulence-associated effector protein gene avrA is dependent on a *Salmonella* enterica-specific regulatory function. Int. J. Med. Microbiol. 296: 25-38.

Bell, C.A.K. (2002). Foodborne pathogens. Cambridge: CRC Press, Wood Head Publishing 2002.

Black, J.G. (2008). Microbiology: Principles and explorations, New Jersey, John Wiley & Sons 2008.

Borges, K.A., T.Q. Furian, A. Borsoi, H.L Moraes, C.T Salle, and V.P. Nascimento (2013). Detection of virulence-associated genes in *Salmonella* Enteritidis isolates from chicken in South of Brazil. Pesqui Vet Bras. 33: 1416-1422.

Card, R., K. Vaughan, M. Bagnall, J. Spiropoulos, W. Cooley, T. Strickland, R. Davies, and M.F. Anjum (2016). Virulence characterisation of *Salmonella* enterica isolates of differing antimicrobial resistance recovered from UK livestock and imported meat samples. Front. Microbiol. 7: 640.

Castilla, K.S., C.S.A. Ferreira, A.M. Moreno, I.A. Nunes, and A.J.P. Ferreira (2006). Distribution of virulence genes sefC, pefA and spvC in *Salmonella* Enteritidis phage type 4 strains isolated in Brazil. Braz. J. Microbiol. 37: 135-139.

Centers for Disease Control and Prevention. National Enteric Disease Surveillance (CDC): *Salmonella* annual report, 2013. [cited 2019 Jun 27]. Available from: https://www.cdc.gov/nationalsurveillance/pdfs/salmonella-annual-report 2013- 508c.pdf

CLSI, (2007). Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing, 27th ed.; CLSI Supplement M100; CLSI:Wayne, IL, USA; ISBN 1-56238-805-3.

Collier-Hyams, L.S., H. Zeng, J. Sun, A.D. Tomlinson, Z.Q. Bao, H. Chen, J.L. Madara, K. Orth, and A.S. Neish (2002). Cutting edge: *Salmonella* AvrA effector inhibits the key proinflammatory, anti-apoptotic NF-κB pathway. J. Immunol. 169: 2846-2850.

Darwin, K.H., and V.L. Miller (1999). Molecular Basis of the Interaction of *Salmonella* with the Intestinal Mucosa. Clin. Microbiol. Rev. 12: 405-428.

Derakhshandeh, A., R. Firouzi, and R. Khoshbakht (2013). Association of three plasmid-encoded spv genes among different *Salmonella* serotypes isolated from different origins. Indian J Microbiol. 53: 106-110.

El-Sebay, N.A., H. Abu Shady, S. El-Rashed El-Zeedy, and A. Samy (2017). InvA gene sequencing of *Salmonella* Typhimurium isolated from Egyptian poultry. Asian J. Sci. Res. 10:194-202.

El-Sharkawy, H., A. Tahoun, A.E-G.A. El-Gohary, M. El-Abasy, F. El-Khayat, T. Gillespie, Y. Kitade, H.M. Hafez, H. Neubauer, and H. El-Adawy (2017). Epidemiological, molecular characterization and antibiotic resistance of *Salmonella* enterica serovars isolated from chicken farms in Egypt. Gut Pathogens. 9: 1-12.

Elkenany, R., M.M. Elsayed, A.I. Zakaria, S.A. El-Sayed, and M.A. Rizk (2019). Antimicrobial resistance profiles and virulence genotyping of *Salmonella* enterica serovars recovered from broiler chickens and chicken carcasses in Egypt. BMC Vet. Res. 15: 1-9.

ElSheikh, M., E. Abdeen, and A. Ammar (2019). Molecular Detection of Some Virulence Genes of *Salmonella* Serotypes Isolated From Poultry in Egypt. J. Current Veterinary Research. 1: 86-93.

Fashae, K., F. Ogunsola, F.M. Aarestrup, and R.S. Hendriksen (2010). Antimicrobial susceptibility

and serovars of Salmonella from chickens and humans in Ibadan, Nigeria. J. Infect. Dev. Ctries. 4: 484-494.

Fekry, E., A.M. Ammar, and A.E. Hussien (2018). Molecular Detection of InvA, OmpA and Stn Genes in *Salmonella* Serovars from Broilers in Egypt. Alex. J. Vet. Sci. 56.

Foley, S., A. Lynne, and R. Nayak (2008). *Salmonella* challenges: prevalence in swine and poultry and potential pathogenicity of such isolates. J. Anim. Sci. 86: E149-E162.

García, C., J. Soriano, V. Benítez, and P. Catalá-Gregori (2011). Assessment of *Salmonella* spp. in feces, cloacal swabs, and eggs (eggshell and content separately) from a laying hen farm. Poult. Sci. J. 90: 1581-1585.

Groisman, E.A., and H. Ochman (1997). How *Salmonella* became a pathogen. Trends Microbiol. 5: 343-349.

Haider, M., M. Hossain, M. Hossain, E. Chowdhury, P. Das, and M. Hossain (2004). Isolation and characterization of enterobacteria associated with health and disease in Sonali chickens. Bangladesh J. Vet. Med. 2: 15-21.

Halawa, M., A. Moawad, I. Eldesouky, and H. Ramadan (2016). Detection of antimicrobial phenotypes, ß-lactamase encoding genes and class I Integrons in *Salmonella* serovars isolated from broilers. Int. J. Poult. Sci. 15: 1-7.

Hudson, C.R., C. Quist, M.D. Lee, K. Keyes, S.V. Dodson, C. Morales, S. Sanchez, D.G. White, and J.J. Maurer (2000). Genetic Relatedness of *Salmonella* Isolates from Nondomestic Birds in Southeastern United States. J. Clin. Microbiol. 38: 1860-1865.

Huehn, S., R.M. La Ragione, M. Anjum, M. Saunders, M.J. Woodward, C. Bunge, R. Helmuth, E. Hauser, B. Guerra, and J. Beutlich (2010). Virulotyping and antimicrobial resistance typing of *Salmonella* enterica serovars relevant to human health in Europe. Foodborne Pathog Dis. 7: 523-535.

Humphrey, T. (2000). Public-health aspects of *Salmonella* infection. *Salmonella* in domestic animals; 1: 245-263.

International Organization of Standardization (ISO) 6579. Microbiology general guidelines on methods for the detection of Salmonella. International organization of standardization, Geneva, Switzerland 2002.

Kauffman, F. Kauffman White Scheme. WHO, BD 1972, L. Rev., 1 Acta Pathologica et Microbiologica Scandinavica 1974; 61: 385.

Khairy, R.M.M. (2015). Anti-microbial resistance of non-typhoid Salmonella in Egypt. Fermentol Techno. 4:2.

Khallaf, M., N. Ameur, M. Terta, M. Lakranbi, S. Senouci, and M.M. Ennaji (2014). Prevalence and antibiotic-resistance of Salmonella isolated from chicken meat marketed in Rabat, Morocco. Int. J. Innov. Appl. Studies. 6(4): 1123-1128.

Khaltabadi Farahani R., P. Ehsani, M. Ebrahimi-Rad, and A. Khaledi (2018). Molecular detection, virulence genes, biofilm formation, and antibiotic resistance of *Salmonella* enterica serotype enteritidis isolated from poultry and clinical samples. Jundishapur J. Microbiol. 11.

Kim, J.E., and Y. ju Lee (2017). Molecular characterization of antimicrobial resistant non-typhoidal *Salmonella* from poultry industries in Korea. Ir. Vet. J. 70: 1-9.

Libby, S.J., L.G. Adams, T.A. Ficht, C. Allen, H.A. Whitford, N.A. Buchmeier, S. Bossie, and D.G. Guiney (1997). The spv genes on the *Salmonella* dublin virulence plasmid are required for severe enteritis and systemic infection in the natural host. Infect. Immun. 65: 1786-1792.

Ma, M., H. Wang, Y. Yu, D. Zhang, and S. Liu (2007). Detection of antimicrobial resistance genes of pathogenic Salmonella from swine with DNA microarray. J. Vet. Diagn. Investig.19:161–167.

Mathole, M., F. Muchadeyi, K. Mdladla, D. Malatji, E. Dzomba, and E. Madoroba (2017). Presence, distribution, serotypes and antimicrobial resistance profiles of Salmonella among pigs, chickens and goats in South Africa. Food Control, 72: 219-224.

Majowicz, S.E., J. Musto, E. Scallan, F.J. Angulo, M. Kirk, S.J. O'Brien, T.F. Jones, and R.M. Fazil Hoekstra (2010). The global burden of nontyphoidal *Salmonella* gastroenteritis. Clin. Infect. Dis. 50: 882-889.

Marcus, S.L., J.H. Brumell, C.G. Pfeifer, and B.B. Finlay (2000). *Salmonella* pathogenicity islands: big virulence in small packages. Microbes Infect. 2: 145-156.

Medeiros, M.A.N., D.C.N.d. Oliveira, D.d.P. Rodrigues, and D.R.C.d. Freitas (2011). Prevalence and antimicrobial resistance of *Salmonella* in chicken carcasses at retail in 15 Brazilian cities. Revista Panamericana de Salud Pública; 30: 555-560.

Menghistu, H.T., R. Rathore, K. Dhama, and R.K. Agarwal (2011). Isolation, identification and polymerase chain reaction (PCR) detection of *Salmonella* species from field materials of poultry origin. Intl J Microbiol Res. 2: 135-142.

Mezal, E.H., A. Sabol, M.A. Khan, N. Ali, R. Stefanova, and A.A. Khan (2014). Isolation and molecular characterization of *Salmonella* enterica serovar Enteritidis from poultry house and clinical

samples during 2010. Food Microbiol. 38: 67-74.

Moussa, I.M., Y.S. Aleslamboly, A.A. Al-Arfaj, A.M. Hessain, A.S. Gouda, and R.M. Kamal (2013). Molecular characterization of *Salmonella* virulence genes isolated from different sources relevant to human health. J. Food Agric. Environ. 11: 197-201.

Mthembu, T.P., O.T. Zishiri, and M.E. El Zowalaty (2019). Detection and molecular identification of *Salmonella* virulence genes in livestock production systems in South Africa. Pathogens; 8: 124.

Murugkar, H., H. Rahman, and P. Dutta (2003). Distribution of virulence genes in *Salmonella* serovars isolated from man & animals. I Indian J Med Res. 117: 66.

Muthu, G., A. Suresh, D. VishnuPrabu, A. Munirajan, S.E. Mary, E. Sathishkumar, P. Gopinath, and S. Srivani (2014). Detection of virulence genes from *Salmonella* species in Chennai, India. CIB Tech. J. Microbiol. 3: 11-14.

Naik, V.K., S. Shakya, A. Patyal, and N.E. Gade (2015). Detection of virulence genes in *Salmonella* Species isolated from chevon and chicken meat. J. Anim. Res. 5: 115.

Nakano, M., E. Yamasaki, A. Ichinose, T. Shimohata, A. Takahashi, J.K. Akada, K. Nakamura, J. Moss, T. Hirayama, and H. Kurazono (2012). *Salmonella* enterotoxin (Stn) regulates membrane composition and integrity. Dis Model Mech. 5: 515-521.

Norris, F.A., M.P. Wilson, T.S. Wallis, E.E. Galyov, and P.W. Majerus (1998). SopB, a protein required for virulence of Salmonella dublin, is an inositol phosphate phosphatase. Proc Natl Acad Sci USA. 95: 14057-14059.

OIE, (2004). Salmonellosis in manual of diagnostic tests and vaccines for terrestrial animals. ^{5}th Edn., OIE, Paris, France.

Okamoto, A.S., R.L. Andreatti Filho, T.S. Rocha, A. Menconi, and G.A. Marietto-Gonçalves (2009). Relation Between the SpvC and InvA Virulence Genes and Resistance of *Salmonella* enterica Serotype Enteritidis Isolated from Avian Material. Int. J. Poult. Sci. 8: 579-582.

Okeke, I., R. Laxminarayan, Z. Bhutta, A. Duse, P. Jenkins, T. O'Brien, A. Pablos-Mendez, and K.P. Klugman (2005). Antimicrobial resistance in developing countries. Part I: recent trends and current status. Lancet. Infect. Dis. 5 (8):481-493.

Oliveira, S., C. Rodenbusch, M. Ce, S. Rocha, and C. Canal (2003). Evaluation of selective and non-selective enrichment PCR procedures for *Salmonella* detection. Lett. Appl. Microbiol. 36: 217-221.

Rabie, N.S., N.O. Khalifa, M.E. Radwan, and J.S. Afify (2012). Epidemiological and molecular studies of *Salmonella* isolates from chicken, chicken meat and human in Toukh, Egypt. Glob. Vet. 8: 128-132.

Rahman, H. (2006). Prevalence & phenotypic expression of sopB gene among clinical isolates of *Salmonella* enterica. Indian J. Med. Res. 123: 83.

Ramatla, T., M.O. Taioe, O.M. Thekisoe, and M. Syakalima (2019). Confirmation of antimicrobial resistance by using resistance genes of isolated *Salmonella* spp. in chicken houses of north west, South Africa. World; 9: 158-165.

Rostagno, M., I. Wesley, D. Trampel, and H. Hurd (2006). *Salmonella* prevalence in market-age turkeys on-farm and at slaughter. Poult. Sci. 85: 1838-1842.

Rotger, R., and J Casadesús (1999). The virulence plasmids of *Salmonella*. Int Microbiol. 2: 177-184.

Roudier, C., J. Fierer, and D. Guiney (1992). Characterization of translation termination mutations in the spv operon of the *Salmonella* virulence plasmid pSDL2. J. Bacteriol. 174: 6418-6423.

Salehi, T.Z., M. Mahzounieh, and A. Saeedzadeh (2005). Detection of invA gene in isolated *Salmonella* from broilers by PCR method. Int. J. Poult. Sci. 4: 557-559.

Samanta, I., S. Joardar, P. Das, T. Sar, S. Bandyopadhyay, T. Dutta, and U. Sarkar (2014). Prevalence and antibiotic resistance profiles of *Salmonella* serotypes isolated from backyard poultry flocks in West Bengal, India. J Appl Poult Res. 23: 536-545.

Siriken, B., H. Türk, T. Yildirim, B. Durupinar, and I. Erol (2015). Prevalence and characterization of Salmonella isolated from chicken meat in Turkey. J. Food Sci. 80 (5):M1044–1050.

Skyberg, J.A., C.M. Logue, and L.K. Nolan (2006). Virulence genotyping of *Salmonella* spp. with multiplex PCR. Avian Dis. 50: 77-81.

Srinivasan, P., G.A. Balasubramaniam, T.R. Gopala, K. Murthy, S. Saravanan, and P. Balachandran (2014). Prevalence and Pathology of Salmonellosis in Commercial Layer Chicken from Namakkal, India. Pakistan Vet. J. 34.

Suez, J., S. Porwollik, A. Dagan, A. Marzel, Y.I. Schorr, P.T. Desai, V. Agmon, M. McClelland, G. Rahav, and O. Gal-Mor (2013). Virulence gene profiling and pathogenicity characterization of non-typhoidal *Salmonella* accounted for invasive disease in humans. PloS One; 8: e58449.

Tarabees, R., G. Helal, and G. Younis (2019). Molecular Characterization of Virulence Genes Associated with *Salmonella* spp. Isolated From Poultry. J. Current Veterinary Research. 1: 36-46.

Thung, T.Y., S. Radu, N.A. Mahyudin, Y. Rukayadi, Z. Zakaria, N. Mazlan, B.H. Tan, E. Lee, S.L. Yeoh, Y.Z. Chin, and C.W. Tan (2018). Prevalence, virulence genes and antimicrobial resistance profiles of Salmonella serovars from retail beef in Selangor, Malaysia. Front. microbiol. 8: 2697.

Yang, B., M. Xi, X. Wang, S. Cui, T. Yue, H. Hao, Y. Wang, Y. Cui, W. Alali, and J. Meng (2011). Prevalence of *Salmonella* on raw poultry at retail markets in China. J Food Prot. 74: 1724-1728.

Zhao, X., C. Ye, W. Chang, and S. Sun (2017). Serotype distribution, antimicrobial resistance, and class 1 integrons profiles of Salmonella from animals in slaughterhouses in Shandong province, China. Front. microbiol. 8: 1049.

Zishiri, O.T., N. Mkhize, and S. Mukaratirwa (2016). Prevalence of virulence and antimicrobial resistance genes in *Salmonella* spp. isolated from commercial chickens and human clinical isolates from South Africa and Brazil. Onderstepoort J. Vet. Res. 83: 1-11.