Antibiotic Resistance of *Salmonella* in Poultry Farms of Mauritius

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ABSTRACT

The increased prevalence of *Salmonella* contamination in poultry has gained considerable scientific attention during the last few decades. Poultry is one of the most common reservoirs of *Salmonella* and contamination of poultry products can occur during the different stages of poultry production. The purpose of the study was to determine the prevalence of antibiotic resistant *Salmonella* in poultry and poultry products in Mauritius. Thirty poultry samples were analyzed for *Salmonella* using traditional culturing, serological and PCR assays. The isolates were then tested for resistance against five antibiotics (ampicillin, chloramphenicol, erythromycin, streptomycin and tetracycline) using the disc diffusion susceptibility test. Serotyping showed positive agglutination for *Salmonella* using polyvalent Anti-O and Anti-H antisera. Out of the 30 samples tested, only 5 samples were confirmed as *Salmonella*. It was found that 72% of isolates were resistant to at least one antibiotic. The frequency of antibiotic resistance ranked in the following order: tetracycline (100%), erythromycin (80%), streptomycin (80%), chloramphenicol (60%) respectively. However, 2 out of 5 isolates were susceptible to ampicillin. The findings of this study strongly indicated that antibiotic resistance patterns of *Salmonella* spp. observed in this study are comparable to patterns of other countries.
Key words: *Salmonella*, Poultry, Antibiotic, Resistance, Pattern

INTRODUCTION

Salmonellosis is one of the most common foodborne illnesses worldwide that caused 2.8 billion cases of gastroenteritis annually and severe economic losses (Majowickz et al., 2010). Foods of animal origin such as poultry, eggs and dairy products are mainly involved in the outbreak of human salmonellosis (Linam et al., 2007). The fecal-oral route is known to be the major route of transmission of *Salmonella* from the environment to humans. In poultry, *Salmonella* contamination occurs through vehicles such as poultry feed, air, litter and unhygienic conditions, and vectors, such as insects, rodents and humans (Jones et al., 1991).

In general, control of *Salmonella* is rather difficult since contamination occurs from raw to finished product during various stages of chicken processing. The use of antimicrobial chemotherapy to control salmonellosis in animal husbandry has resulted in resistant microorganisms (Zhao et al., 2007) including *Salmonella*. Besides human use, antibiotics have numerous applications in the veterinary and agricultural field. A large number of antibiotics are fed to animals in farms for prophylactic and therapeutic purposes. Concerns about infections due to *Salmonella* have led to the implementation of control programs in the European Union (EU) for broiler flocks (*Gallus gallus*) (EFSA, 2011). In 2006, the European Union officially banned the use of antibiotics added to poultry feeds as growth promoting substances in livestock (WHO, 2011). In Mauritius, little information is available on the resistance patterns of *Salmonella* in

animal husbandry. Therefore, this study was conducted to determine the prevalence of *Salmonella* spp. and patterns of drug resistance of *Salmonella* isolates in poultry and poultry products.

MATERIAL AND METHODS

Sample collection

Samples of poultry intestine (7), gut (7), egg (9) and litter of two different farms (7) were collected from poultry farms of different regions of Mauritius totaling 30 samples. At least 25 g of each sample were collected and placed in sterile stomacher bags. The samples were kept fresh at room temperature before analysis. Microbiological analysis was carried out within 2hours of sample receipt.

Isolation procedure

Twenty-five grams of each sample was weighed, to which 225ml of 1% buffered peptone water in a sterile stomacher bag. The mixture was shaken and incubated at 37°C for 24 hours. The samples were enriched in selective broth Rappaport Vassiliadis Soya (RVS) and incubated on 37°C for 24 hours. A loopful of the material from the RVS broth was transferred and streaked onto Xylose Lysine Deoxycholate agar (XLD). The plates were inverted and incubated at 37°C for 24 hours. Gram staining was performed to identify any presumptive colonies for *Salmonella*. Out of the 30

samples analyzed, presumptive *Salmonella* spp was isolated from samples of eggs (1), intestine (1), gut (5) and chicken litter (2) originating from different farms.

Molecular identification

DNA extraction and Polymerase chain reaction

The protocol used to prepare genomic DNA was adapted from the method used by Hai-Rong and Ning (2006). A total of nine suspected *Salmonella* isolates were tested by Polymerase Chain Reaction (PCR). The master mix was prepared in the Eppendorf tube, which contained all the reagents except the DNA. The tubes were then spun quickly. Prior to adding 2μl of DNA, 25μl of reagents were added to each PCR tubes. Subsequently, the PCR tubes were run in a thermocycler. PCR was carried out using forward primer Salm3 5'-TATCGCCACGTTCGGGCAA-3') and reverse primer Salm4 (5'-TCGCACCGTCAAAGGAACC-3') targeting the *invA* gene in *Salmonella* (Rahn et al., 1992). The optimized PCR thermocycling conditions were: initial denaturation at 94°C for 15seconds followed by 35 cycles: denaturation at 94°C for 3seconds, primer annealing at 50°C for 10seconds and extension at 74°C for 35seconds; an additional cycle at 74°C for 2 min and 45°C for 2seconds and it was maintained at 4°C until further analysis (Wang et al., 1997). The amplified PCR product was then electrophoresed on 1.5% of agarose gel and the DNA size was determined with 100 bp DNA molecular weight ladder. The wells were loaded with 7μl of PCR product and 2μlbromophenol blue. The gel was run for 2 hours at a constant 90V. Lastly, the gel was viewed under Ultra Violet illumination and the image was captured.

Biochemical tests

Only five isolates (I1-I5) out of nine were PCR identified as *Salmonella*. A series of biochemical tests, namely, citrate, Triple Sugar Iron (TSI), methyl red, Voges–Proskauer, motility, urease, indole, and carbohydrate fermentation tests of glucose, mannitol, sorbitol, arabinose and lactose were subsequently performed on these five isolates. Biochemical test reactions were incubated at 37°Cfor 24-48 hours.

Serological confirmation

Serological assays were conducted on the *Salmonella* isolates using polyvalent anti-O and anti-H antisera (Wallace and Hammack, 2011).

Antibiotic susceptibility testing

Disk diffusion technique was used to test for the susceptibility of *Salmonella* to antibiotics. Three colonies of *Salmonella* were transferred from the XLD agar to 10ml of peptone water. After 24 hours of incubation, 0.1ml of the broth was then spread onto Mueller Hinton agar using sterile spreader. Each disk was placed using sterile forceps and was distributed evenly in the plate and pressed gently to ensure contact with the agar. The susceptibility against the following antibiotics; ampicillin (10μg), chloramphenicol (30 μg), erythromycin (15 μg), streptomycin (10 μg) and tetracycline (30 μg) were determined. The test was carried out within 15 minutes. Only 5 disks were placed in 100 mm of petri plates in order to prevent overlapping of zone of inhibition. Each plate was examined after 16 to 18 hours of incubation. The diameter of zones of inhibition was measured to the nearest whole millimeter using a ruler.

RESULTS AND DISCUSSION

It is widely accepted that *Salmonella* contamination in poultry and poultry products at various stage of production are one of the major factors leading to foodborne illnesses in human and animals. In this study, presumptive *Salmonella* was isolated from nine poultry samples–gut (5), egg (1), intestine (1), litter (2) - using traditional culturing method.

Since all *Salmonella* species possess the *invA* gene, it is used as a marker to detect the presence of *Salmonella* in any sample (Jordan et al., 2009). Following PCR amplification of the nine samples, only five yielded PCR products indicative of the presence of the *invA* gene. The band size obtained after PCR confirmation was ~300 bp corresponding to the size of *invA* gene (Figure 1). Isolates from gut, egg, intestine and two different litter samples were termed I1, I2, I3, I4 and I5 respectively.
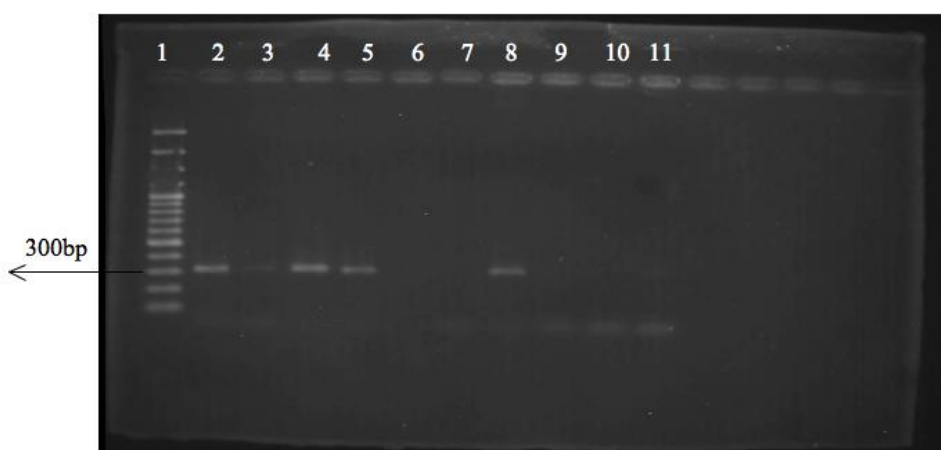

Figure 1. Agarose gel electrophoresis for genomic DNA extracted from nine *Salmonella* isolates. (Lane 1: Molecular marker of 100bp; Lane 2: DNA samples from poultry intestine; Lane 3: DNA samples from egg; Lane 4 and 5: DNA samples from litter; Lane 6: negative control; Lane 7 - 11: DNA samples from poultry gut)

All five isolates were further confirmed by biochemical and serological tests for *Salmonella*. Results of biochemical tests are summarized in Table 1. With respect to the carbohydrate fermentation pattern of the isolates, all the isolates were able to ferment glucose, mannitol, sorbitol, arabinose, but not lactose. The carbohydrate fermentation profile of the five isolates is summarized in Table 2.

Table 1. Biochemical test results of five *Salmonella* isolates of poultry origin

Biochemical Tests	Test results of *Salmonella* isolates				
	I1 (Gut)	I2 (Egg)	I3 (Intestine)	I4 (Litter 1)	I5 (Litter 2)
Citrate	+	+	+	+	+
Triple Sugar Iron	+	+	+	+	+
Methyl Red	+	+	+	+	+
Voges-Proskauer	+	+	+	+	+
Urease	-	-	-	-	-
Indole	-	-	-	-	-
Motility	+	+	+	+	+

Table 2. Carbohydrate fermentation profiles of *Salmonella* isolates in poultry farms, Mauritius

Carbohydrate	Carbohydrate fermentation activity of *Salmonella* isolates				
	I1 (Gut)	I2 (Egg)	I3 (Intestine)	I4 (Litter1)	I5 (Litter 2)
Glucose	+	+	+	+	+
Lactose	-	-	-	-	-
Arabinose	+	+	+	+	+
Mannitol	+	+	+	+	+
Sorbitol	+	+	+	+	+

Serological confirmation relies on agglutination reactions between antigens and antibodies. Agglutination was observed against anti-O and anti-H antisera indicating the presence of O and H antigens (Figure 2).
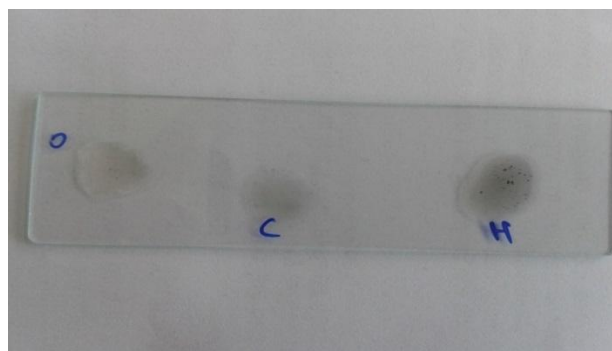

Figure 2. Positive agglutination test in the presence of Anti-O (left) and Anti-H (right) antisera but no agglutination in control (middle).

Among the samples examined 17% were found to be positive for *Salmonella*, which was found to be higher than cases reported in Brazil (2.7%) (Medeiros et al., 2011) or Thailand (4%) (Padungtod et al., 2006). However, Antunes et al. (2003) reported that occurrence of *Salmonella* isolated from poultry samples were 60% in Portugal (Maharjan et al., 2006) which were higher than reported in the present study. The variation in the prevalence of *Salmonella* may be due to the following reasons; sample size as well as the large variations in the sanitary conditions prevailing in the farms. The seasons during which the study was carried out might be another factor since it was found that summer seasons favor the occurrence of *Salmonella* in Mauritius. Similarly, Maharjan et al. (2006) found that prevalence of *Salmonella* was highest during the months of April and May in Nepal (Maharjan et al., 2006). Also, numerous studies used different media for enrichment, selective enrichment and isolation of the pathogen, which are thought to affect the sensitivity of the detection method for *Salmonella* (Carli et al., 2001; Nesa et al., 2011).

It was found that 72% (18 out of 25) of all *Salmonella* isolates were resistant to all five antibiotics tested, hence highlighting the preponderance of multidrug-resistant *Salmonella* in poultry in Mauritius. All isolates were resistant to tetracycline while 60 and 80% of isolates were resistant to chloramphenicol and erythromycin respectively. The isolates also demonstrated varying level of sensitivity to the drugs ranging from 'susceptible', 'intermediate' and

'resistant'. The antibiotic sensitivity patterns of the isolates are shown in Figure 3. Overall, 12 % of the isolates were found to have intermediate resistance and 16 % of isolates were found to be susceptible.
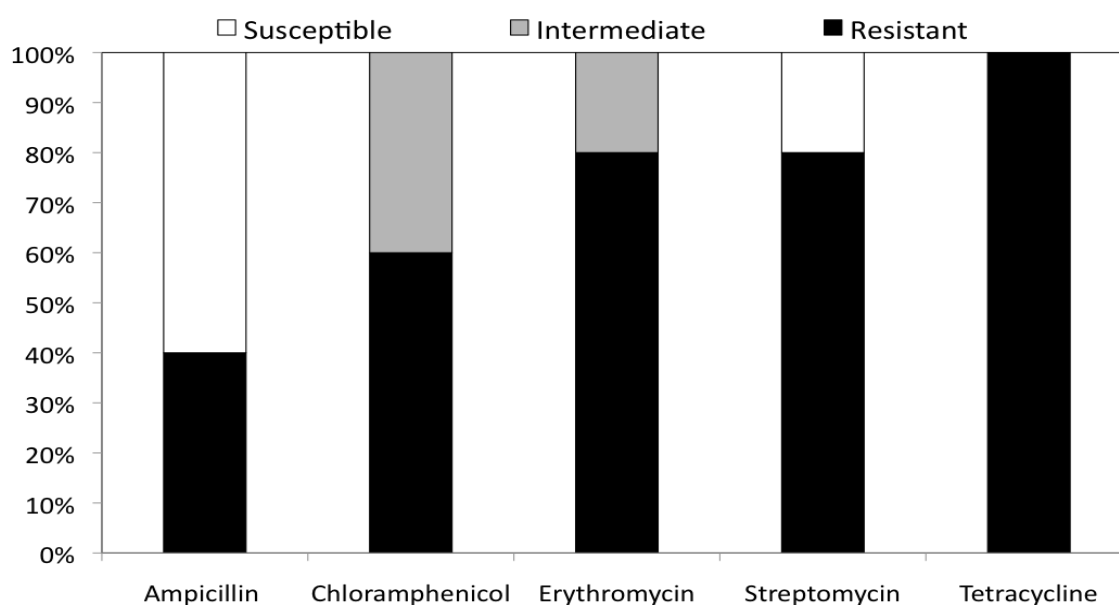

Figure 3. Antibiotic susceptibility patterns of *Salmonella* isolates in poultry farms, Mauritius

Disk diffusion testing is one of several phenotypic assays, which can be utilized to determine the antimicrobial resistant profile (antibiogramme) of an organism. Disk diffusion tests thus give a measure of in-vitro susceptibility. *Salmonella* isolates were categorized as resistant, intermediate or susceptible if the diameter of the inhibition zones were ≤11, 12-14 or ≥14 mm respectively. All isolates were resistant to at least one antibiotic (i.e. zone diameters of ≤11mm). However, no zone of inhibition was observed for I4 and I5 (poultry litter) in response to ampicillin and tetracycline and I1 (poultry gut) with tetracycline. This implies that these strains were highly resistant to the drug. It was observed that I2 (eggs) and I3 (poultry intestine) were moderately resistant to erythromycin while I1 (poultry gut) was moderately resistant to streptomycin. In contrast, I1, I2 and I3 were susceptible to ampicillin.

Table 3 summarizes the degree of sensitivity of the five *Salmonella* isolates to different antibiotics. Other authors have similarly reported that *Salmonella* isolated from pigs were resistant to several antimicrobials, including streptomycin and tetracycline but were sensitive to ampicillin (Gebreyes et al., 2000; Farrington et al., 2001; Gebreyes and Altier, 2002; Rajic et al., 2004; Sisak et al., 2004). This could be partly attributed to the inadequate dose, extensive use, and sub-active concentration of the drug used in poultry farms (Davis, 1994). Furthermore, widespread use of antibiotics in medical, veterinary, agricultural and aquacultural settings as prophylactic measures and growth promoters have resulted in resistance to a large spectrum of antibiotics leading to the proliferation of antibiotic resistant genes in the horizontal gene pool (Meervenne et al., 2012).

Table 3. Antibiotic susceptibility of *Salmonella* in poultry farms, Mauritius

Salmonella Isolates	Diameter of Inhibition Zones (mm)				
	Ampicillin	**Chloramphenicol**	**Erythromycin**	**Streptomycin**	**Tetracycline**
I1 (Gut)	30	16	7	14	6
I2 (Egg)	30	10	14	10	12
I3 (Intestine)	27	15	13	11	10
I4 (Litter 1)	6	12	4	9	6
I5 (Litter 2)	6	10	8	7	6

Overall, highest frequency of resistance was observed with tetracycline (100%) followed by erythromycin (80%) and streptomycin (80%). One likely explanation for the high resistance to tetracycline

and erythromycin could be because of their low cost, ready availability and ease of administration, rendering them more prone to misuse. In Nigeria, streptomycin resistance ranges from 71 to 79% (Sosa et al., 2010),

which is comparable to the findings of this study. Moreover, these antibiotics have been customarily used in poultry to control salmonellosis and are thought to have a broad spectrum of antibacterial activity (Manie et al., 1998). Resistance to tetracycline has been attributed to several genetic determinants associated with mobile plasmids or transposons. Though chloramphenicol and streptomycin are not commonly used for veterinary applications, more than 50% of the isolates displayed resistance to these drugs. This may be due to the fact that the antibiotic resistance genes have altered the microbial community by continuous antibiotic usage and the effects still persist for years even after discontinued use (Sommer and Dantas, 2011). It has been reported that chloramphenicol efflux pumps and chloramphenicol acetyltransferase activity are encoded in the *cmlA* (Cabrera et al., 2004) and *floR* (White et al., 2001) genes of *Salmonella*. The frequency of chloramphenicol resistance in most European countries is reported to be lower than 10%. However, in Greece 40% of isolates of *Salmonella* spp. were resistant to this antibiotic in 2007. The resistance level to chloramphenicol ranged between 13% and 38% from 2004 to 2007 (EFSA, 2010). In India, 100% sensitivity was observed in chloramphenicol for *Salmonella* isolates (Ahmed et al., 2011). In the current investigation, 60% sensitivity to ampicillin was observed; similarly about 56% was reported in Costa Rica (Sosa et al., 2010). On the other hand, 100% resistance to this antibiotic was noted in Egypt (El-Jakee et al., 2010). In Brazil, 3% ampicillin resistance was detected in *S*. Enteritidis and 10% in Chile (Sosa et al., 2010). One possible explanation for this finding is that ampicillin first binds to Penicillin-Binding Proteins (PBPs) and thus interferes with the formation of cell wall, which proved to be effective against the organisms. Interestingly, the isolates presented in the research exhibited drug resistances against all five antibiotics tested. The findings also revealed that frequencies of multi-drug resistance among *Salmonella* isolated were 100%. Similarly other findings reported in poultry were 100% in Turkey (Dogru et al., 2010), 100% in Nepal (Shrestha et al., 2010), 92% in USA (Zhao et al., 2005), 80% in China (Yang et al., 2010) and 75% Portugal (Antunes et al., 2003) while 2.3% was described in Southern Italy which is far higher (Nastasi et al., 2000). The indiscriminate use of antimicrobials in livestock farming have resulted in increased resistance and these have been transmitted to humans via the food chain and thus have been the major cause of drug resistance in humans. Also, resistant strains not only hinder treatment, but are also found to cause more severe illnesses in humans (Holmberg et al., 1984).

CONCLUSION

It can be inferred that the trends of antimicrobial resistance of *Salmonella* observed across the world are reflected in this study. It is necessary to closely monitor the hygienic practices prevailing in farms and the food production systems to minimize or eliminate the risk of antibiotic-resistant bacteria in the food chain. Despite low incidence of *Salmonella* reported in this work, yet a high proportion of resistance was recorded. Moreover, an upsurge in the percentage of *Salmonella* isolates exhibiting single-drug resistance or multi-drug resistance can hinder human and animal therapy and hence the surveillance of antibiotic resistance should be intensified in Mauritius.

REFERENCES

Ahmed MM, Rahman MM, Mahbub KR and Wahiduzzaman M (2011). Characterization of antibiotic resistant *Salmonella* spp isolated from chicken eggs of Dhaka City. Journal of Scientific Research, 3(1): 191-196.

Antunes P, Reu C, Sousa JC, Peixe Land Pestana N (2003). Incidence of *Salmonella* from poultry products and their susceptibility to antimicrobial agents. International Journal of Food Microbiology, 82: 97-103.

Cabrera R, Ruiz J, Marco F, Oliveira I, Arroyo M, Aladuena A, Usera MA, Jimenez De Anta, MT, Gascon J and Vila J(2004). Mechanism of resistance to several antimicrobial agents in *Salmonella* clinical isolates causing traveler's diarrhea. Antimicrobial Agents Chemotherapy, 48: 3934-3939.

Carli KT, Eyigor A and CanerV (2001). Prevalence of *Salmonella* serovars in chickens in Turkey. Journal of Food Protection, 64: 1832-1835.

Davis J (1994). Inactivation of antibiotics and the dissemination of resistance genes. Journal of Food Science, 264: 375-382.

Dogru AK, Ayaz ND and Gencay YE (2010). Serotype identification and antimicrobial resistance profiles of *Salmonella* spp. isolated from chicken carcasses. Tropical Animal Health and Production, 42: 893-897.

European Food Safety Authority (2011). The European Union summary report on trends and sources of zoonoses, zoonotic agents and foodborne outbreaks in 2009. European Food Safety Authority Journal, 9: 2090-2477.

European Food Safety Authority (2010). Antimicrobial resistance in zoonotic agents from animals and food in the European Union in 2004 - 2007. European Food Safety Authority Journal, 8: 1309-1310.

El-Jakee J, Abd El-Moez SI, Mohamed KF, Effat MM, Samy AA and Gad El-Said WA(2010). Restriction enzyme, plasmid profile analysis and antibiotic resistance of *Salmonella* Typhimurium of poultry origin isolated from Egyptian Farms. International Journal of Microbiology, 1: 137-146.

Hai-Rong C and Ning J (2006). Extremely rapid extraction of DNA from bacteria and yeasts. Biotechnology Letters, 28 (1): 55-59.

Holmberg SD, Wells JG and Cohen ML (1984). Animal-to-man transmission of antimicrobial-resistant *Salmonella*: investigations of U.S. outbreaks, 1971- 1983. Science, 225: 833-835.

Jones FT, Axtell RC, Rives DV, Scheideler SE, Tarver J, Walker RL and Wineland MJ (1991). A survey of *Salmonella* contamination in modern broiler production. Journal of Food Protection, 54: 502-507.

Jordan R, Van Heerden E, Hugo CJ and Piater LA (2009). Using current molecular techniques for rapid differentiation of *Salmonella* Typhi and *Salmonella* Typhimurium. African Journal of Biotechnology, 8 (9): 1815-1818.

Kariuki S (2010). Antimicrobial resistance in enteric pathogens in developing countries. In: A. Sosa et al. (Editors), Antimicrobial resistance in developing countries. Springer, New York, pp. 177-199.

Linam WM and Gerber MA (2007). Changing epidemiology and prevention of *Salmonella* infections. The Pediatric Infectious Disease Journal, 26 (8): 747-749.

Maharjan M, Joshi V, Joshi DD and Manandhar P (2006). Prevalence of *Salmonella* species in various raw meat samples of a local market in Kathmandu. Trends in the Study of Disease Agents, 1081: 249-256.

Majowicz SE, Musto J, Scallan E, Angulo FJ, Kirk M, O'brien SJ, Jones TF, Fazil A and Hoekstra RM (2010). The Global Burden of Nontyphoidal *Salmonella* Gastroenteritis. Clinical Infectious Diseases, 50(6): 882-889.

Manie T, Khan S, Brozel VS, Veith, WJ and Gouws PA (1998). Antimicrobial resistance of bacteria isolated from slaughtered and retail chickens in South Africa. Letters in Applied Microbiology, 26: 253-258.

Medeiros MA, Oliveira DC, Rodrigues P and Freitas DR (2011). Prevalence and antimicrobial resistance of *Salmonella* in chicken carcasses at retail in 15 Brazilian cities. The Revista Panamericana de Salud Pública, 30: 555-560.

Meervenne EV, Coillie EV, Kerckhof FM, Devlieghere F, Herman L, De Gelder LSP, Top EM and Boon N (2012). Strain specific transfer of antibiotic resistance from an environmental plasmid to foodborne pathogens. Journal of Biomedicine and Biotechnology. Article ID 834598, 8 pages.

Nastasi A, Mammina C and Cannova L (2000). Antimicrobial resistance of *Salmonella* Enteritidis in Southern Italy, 1990-1998. Emerging Infectious Diseases, 6: 401-403.

Nesa MK, Khan MSR and Alam M (2011). Isolation, identification and characterization of *Salmonella* serovars from diarrhoeic stool samples of human. Journal of Veterinary Medicine, 9(1): 85-93.

Padungtod P and Kaneene JB (2006). *Salmonella* in food animals and humans in Northern Thailand. International Journal of Food Microbiology, 108: 346-354.

Rahn K, DeGrandis SA, Clarke RC, Curtiss R and Gyles CL (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: 271-279.

Shrestha A, Regmi P, Dutta RK, Khanal DR, Aryal SR, Thakur RP, Karki D and Singh UM (2010). First report on antimicrobial resistance of *Salmonella* isolated from poultry in Nepal. Veterinary Microbiology, 144: 522-524.

Sommer MOA and Dantas G (2011). Antibiotics and the resistant microbiome. Current Opinion in Microbiology, 14: 556–563

Wallace HA and Hammack TS (2011). *Salmonella*. Bacteriological Analytical Manual, Center for Food Safety and Applied Nutrition, U.S. Food and Drugs Administration.

Wang RF, Cao WW and Cerniglia CE (1997).A universal protocol for PCR detection of 13 species of foodborne pathogens in foods. Journal of Applied Microbiology, 83: 727–736.

White DG, Zhao S, Sudler R, Ayers S, Freidman S, Chen S and Meng J (2001). The isolation of antibiotic-resistant *Salmonella* from retail ground meats. New England Journal of Medicine, 345(16): 1147-1154.

WHO (2011). Tackling antibiotic resistance from a food safety perspective in Europe. Available from: http://www.euro.who.int/data/assets/pdf_file/0005/1364 54/e94889.pdf.

Yang B, Qu D, Zhang X, Shen J, Cui S, Shi Y, Xi M, Sheng M, Zhi S and Meng J (2010). Prevalence and characterization of *Salmonella* serovars in retail meats of marketplace in Shaanxi, China. International Journal of Food Microbiology, 141: 63-72.

Zhao S, Fedorka-Cray PJ, Freidman S, Mcdermott PF, Walker RD, Qaiyumi S, Foley SL, Hubert SK, Ayers S, English L, Dargatz DA, Salamone B and White DG (2005). Characterization of *Salmonella* Typhimurium of animal origin obtained from the national antimicrobial resistance monitoring system. Foodborne Pathogens and Disease, 2:169-181.

Zhao S, Mcdermott, PF, White DG, Qaiyumi S, Freidman SL, Abbott JW, Glenn A, Ayers SL, Post KW, Fales WH, Wilson RB, Reggiardo C and Walker RD (2007). Characterization of multidrug resistant *Salmonella* recovered from diseased animals. Veterinary Microbiology, 123: 122-123.