INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print), 2222-5234 (Online)
http://www.innspub.net
Vol. 14, No. 1, p. 284-292, 2019

RESEARCH PAPER OPEN ACCESS

Isolation, identification and antibiotic susceptibility pattern of *Salmonella* species isolated from poultry samples of District Kohat

Inam Ullah[1*], Nasir Mahmood Khattak[2], Zahidullah Khan[3], Hazir Rehman[1], Hassan Naveed[1], Malik Nawaz Shuja[1], Muhammad Naeem[4], Matiullah[5], Ikram Ullah[6], Hameed Ur Rehman[6]

[1]*Department of Microbiology, Kohat University of Science and Technology, KPK, Pakistan*

[2]*College of Biological Sciences Northeast Forestry University, No.26 Hexing Road Xiangfang District Harbin Heilongjiang Province, China*

[3]*Assistant Professor (Microbiology) Pathology Department Khyber Girls Medical College Peshawar.*

[4]*Directorate of Cereal Crops Agriculture Research Institute ARI, Sariab Quetta, Pakistan*

[5]*Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University Islamabad, Pakistan*

[6]*Department of Chemistry, Kohat University of Science and Technology, KPK, Pakistan*

Key words: Isolation, Identification, Antibiotic Susceptibility, *Salmonella species*, Poultry Samples, District Kohat.

http://dx.doi.org/10.12692/ijb/14.1.284-292 Article published on January 26, 20199

Abstract

Salmonella species has been recognized as a major cause of food borne illness associated with poultry worldwide. Salmonellosis is one of the most common food borne diseases in industrial and developing countries. The wide spread of antimicrobial-resistance in *Salmonella species* has been a serious global human and animal health problem. The aims of this study were to estimate the prevalence and antimicrobial susceptibility pattern of *Salmonella species* from poultry samples of District Kohat. The isolates were screened for antimicrobial susceptibility pattern by using disk diffusion method. Out of 100 poultry samples, 35 were positive for *Salmonella species*. Most *Salmonella species* were resistant to Ampicillin (n=28; 80%) and Tetracycline (n=27; 78%).40% (n=14) were susceptible to Azithromycin, 31.42% (n=11) were intermediate and 28.57% (n=10) were resistant to Azithromycin 37.14%. Chloramphenicol showed good antibacterial activity against *Salmonella species*. 18 isolates (51.42%) were sensitive to Chloramphenicol, while 37.14% (n=13) were resistant. Ciprofloxacin and Levofloxacin exhibited poor activity against *Salmonella species* and most of them were intermediate sensitive to Ciprofloxacin (n=18; 51.52%) and Levofloxacin (n=19; 54.28%).High levels of resistance to antibiotics that are used commonly for human and poultry can be a warning for our community health and this information must be used to form important strategies for improvement of infection control.

* **Corresponding Author:** Inam Ullah ✉ Inamullahwazir13@gmail.com

Introduction

Salmonella is comprised of a large circular chromosome consisting of approx. 4.8 mega bases (Mb). Extra-chromosomal DNA can be present in form of plasmids having various sizes. Until now 26 whole genomic *Salmonella* sequences are available [D. A. Benson,2008 1].Essential proteins involved in metabolic pathways are 14 while 8 metabolic pathways are found to be present exclusively in the pathogen comprising of 27 enzymes unique to the pathogen. Thus, these 27 proteins may serve as prospective drug targets [S. Porwollik,2003 2].*Salmonella* generates type 1 fimbriae, the most widely used type of fimbrial mechanism [J. P. Duguid,19663]. Type 1 fimbriae expressed by serovar *typhimurium* were shown to cause persistent infection in swine [C. Althouse,2003 4]. Over 80% of *Salmonella enterica* isolates encode and express this type of fimbriae suggesting that type 1 fimbriae play an important role in some stages of *Salmonella* invasion and life cycle[Y. Chuang,20085]. *Salmonella* is transmitted to vectors such as rats, flies and birds where *Salmonella* can shed in their faeces for weeks and even months. Animals such as swines, cows and chickens act as the important risk factor for infection. Human get infected when eating the food or drinking the water that is contaminated with *Salmonella* through animal reservoirs. *Salmonellatyphi* and *Salmonellapara typhi* A do not have animal reservoir, therefore infection can be caused by eating the improperly handled food by infected individuals [D. G Newell, 20106]. *Salmonella pullorum* and *Salmonella gallinarum* usually cause disease in poultry [J. M. Cox, 20107]. Spectrum of clinical diseases is caused by food borne pathogen *Salmonella* (salmonellosis). Enteric fever is caused by Salmonella typhi whereas paratyphoid fever is caused by Paratyphi A, B and C. Both Salmonella typhi and Salmonella Paratyphi are human pathogens. Bacteremia caused by *Salmonella* should be taken into account in cases of fever of unknown origin [N. R. Thomson, 2008]. Non-typhoidal salmonellosis or enterocolitis is caused by at least 150 *Salmonella* serotypes with *Salmonella typhimurium* and *Salmonella enteritidis* being the most common serotypes. Factors contributing to the chronic carrier state have not been fully explained. Culturing is the most accurate method of diagnosis for Salmonella. The most commonly used media that is selective to *Salmonella species* are SS agar, Bismath sulphite agar, Hektoen enteric medium, Xylene lysine deoxycholate agar and Brilliant green agar [K. Todar, 2005].*Salmonella specie* can also be diagnosed by PCR using blood as a sole source of template DNA of *Salmonella species* [A. V. Kumer, 2002].About 8% of the untreated cases of Salmonellosis result in bacteremia. About 2 to 5% of untreated typhoid infections result in a chronic carrier state. Up to 10% of untreated convalescent typhoid cases will excrete *Salmonella typhi* in feaces for 1 to 3 months and between 1 and 4% become chronic carriers [C. A. Scherer, 2001].Typhoid fever usually causes mortality in 5 to 30% of typhoid-infected individual in the developing world. Typhoid fever is endemic throughout Africa and Asia as well as persists in the Middle East, some eastern, southern European countries, Central and South America. The most famous outbreak of enteric fever is Typhoid Mary [C. A. Scherer, 2001].The aim of the current work was to find out the Isolation, Identification and Antibiotic Susceptibility Pattern of *Salmonella species* Isolated from Poultry Samples of District Kohat.

Materials and methods

A total of 100 poultry samples were collected from different areas of District Kohat (Table 1). The samples were collected from four major areas of district Kohatthat include highway bypass, college town, Kohat main city and Kohat Development Authority (KDA).Samples were aseptically cut, tied and placed in sterile plastic bags which were kept on ice in an insulated cooler box and then transported to the laboratory and were processed on the same day.

The samples were first washed with sterile distilled water and then surface sterilized with 3 % bleach, cut and chopped with sterile blades, then each sample was inoculated into tetrathionate broth at a ratio of 1 : 9 i.e. one gram of different poultry samples were

inoculated into 9 ml tetrathionate broth. All samples were incubated at 37 °C for 24 hours.

Selective plating

The samples from the enrichment broth were then plated on bismuth sulphite agar for the isolation and preliminary identification of *Salmonella species*. In this medium freshly precipitated bismuth sulphite acts together with brilliant green as a selective agent by suppressing the growth of coliforms, whilst permitting the growth of *Salmonella*. Sulphur compounds provide a substrate for hydrogen sulphide production, whilst the metallic salts in the medium stain the colony and surrounding medium black or brown in the presence of hydrogen sulphide. The plates were then incubated at 37 °C for 48 hour. On bismuth sulphite agar the plates having black color colonies were suspected to be positive while colonies of other color were considered as negative.

Morphological and biochemical identification

Morphological identification of *Salmonella species* was done by gram staining. One drop of normal saline was dropped on the sterile slide. Loopful of sample was placed on the slide and then it was air dried and heat fixed. Crystal Violet was poured and kept for about 30 seconds to 1 minute and rinsed with water. Gram's iodine was then poured for 1 minute and then washed with water. It was then washed with 95% alcohol or acetone for about 10-20 seconds and rinsed with water. Safranin was then poured for about 1 minute and washed with water. Finally it was air dried and observed under microscope. Pink or red color gram negative rods were suspected to be *Salmonella species*.

For final identification of bacterial isolates biochemical tests were performed. The biochemical tests included were oxidase, catalase, TSI, Motility, indole, citrate and urease for the identification of bacterial isolates on the basis of biochemical tests. Bergey's Manual of Systemic Bacteriology was used as a reference manual [R. N. Krieg, 1984].

Antimicrobial susceptibility patterns

Modified Kirby-Bauer disk diffusion method was used to test the susceptibility of the *Salmonella species* isolates to different antimicrobial agents: Ampicillin (10 µg), Tetracycline (30 µg), chloramphenicol (30 µg), Azithromycin (10), Levofloxacin (5 µg) and Ciprofloxacin (5 µg), The inocula were prepared by growing the various *Salmonella species* on separate agar plates and colonies from the plate were transferred with inoculating loop into 3 ml of normal saline in a test tube. The density of these suspensions was adjusted to 0.5 McFarland standards. McFarland standards were prepared because they are extensively used as turbidity standards for the preparation of suspensions of microorganisms [V. Lorian, 1986].

First Muller-Hinton Agar was prepared, and then standardized inoculums were prepared by using 0.5 McFarland solutions and were spread on media. Different antibiotic disks were placed in each plate with proper spacing using a sterile forcep. Plates were incubated at 37 °C for 24 hours for proper growth. Antibiotics to which organisms were sensitive formed clear zone around it and to which organisms were resistant, they did not form any zone of inhibition, while those that were intermediate formed a little zone of inhibition. The diameter of the zone was measured with the standard zone diameter given in the protocol chart provided by Clinical Laboratory Standard Institute (CLSI) [CLSI, 2012].

Results

Cultural identification of Salmonella species

On bismuth sulphite agar black to brownish color colonies were suspected as *Salmonella species* (Fig. 1). We preceded black and brownish coloured colonies that might be of *Salmonella species*.

Table 1. Distribution of samples.

Sample type	Number (n)
Heart	20
Liver	20
Kidney	20
Chest piece	20
Leg piece	20
Total	100

Morphological identification of Salmonella species

Gram staining results were observed by observing the slide under 100X of microscope. The Bacteria attaining pink colour were considered as Gram negative rods. The *Salmonella species* isolates were Gram negative rods on Gram's staining (Fig. 2).

Biochemical identification of Salmonella species

Oxidase test

Oxidase positive microorganisms produced enzyme cytochrome oxidase that oxidized the phenylene diamine into a deep purple colour.

Table 2. Biochemical identification for *Salmonella species*.

Biochemical test		Results
Oxidase		Negative
Catlase		Positive
Indole		Negative
Urease		Negative
Citrate		Positive
Motility		Positive
TSI	slope	Red and pink
	Butt	Yellow
	H_2S	Positive
	Gas	Differential

Catalase test

Catalase test positive microorganisms produced enzyme catalase which detoxifies hydrogen peroxide by breaking it down into water and oxygen gas due to which bubble formation occurs. Bubble formation indicated positive while no bubble formation as negative.

Triple sugar iron (TSI) test

Bacterial samples were grown on triple iron sugar (TSI) agar. Changes in colour of butt were considered as glucose fermentation and that of the slope as lactose fermentation, blackening of butt as hydrogen sulphide production. Cracks in the media were considered as gas production.

Table 3. Prevalence of *Salmonella species*.

Variables	Total *Salmonella spp (N)*	*Salmonella species* positive n(%)	*Salmonella species* negative n(%)
Specimen			
Heart	20	5(25)	15(75)
Kidney	20	6(30)	14(70)
Liver	20	7(35)	13(75)
Leg piece	20	9(45)	11(55)
Chest piece	20	8(40)	12(60)
Area			
Highway bypass	25	11(44)	14(56)
College town	25	9(36)	16(64)
Kohat city	25	10(40)	15(60)
KDA	25	6(24)	19(76)

Indole test

Indole test is used to differentiate lactose fermantive species and non-lactose fermantive *enterobactericeae* members. A red ring circle formation on the surface of the tube by the addition of Kovac's reagent was considered as positive.

Motility test

Motility test is used to differentiate between motile and non-motile bacteria. Motility positive test was indicated by the presence of diffuse growth (appearing as coloring of the medium) away from the line of inoculation while in motility negative test there

is no diffused growth.

Urease test

Urease test positive organisms are capable of hydrolyzing urea to produce ammonia and carbon dioxide.

Urease positive test is indicated by appearance of bright pink color on the slant surface while no color as urease negative test.

Citrate utilization test

Citrate utilization test was performed to differentiate *Salmonella species* from other Enterobacteriaceae members. If the organism changed the colour of media from green to blue, it was considered to be positive for citrate utilization.

Prevalence of Salmonella species isolates

The overall prevalence of *Salmonella species* in these samples were 35 % (35/100) (Table 3).

Table 4. Antibiotic drugs susceptibility pattern of *Salmonella species*.

S.No	Antimicrobial drugs	Code	Drug susceptibility	*Salmonella species*(n)%
1	Tetracycline(30µg)	TE	*Susceptible*	3 (8.57)
			Intermediate	5 (14.28)
			Resistant	27 (77.14)
2	Azithromycin(15µg)	AZM	*Susceptible*	14 (40)
			Intermediate	11 (31.42)
			Resistant	10 (28.57)
3	Chloramphenicol (30µg)	C	*Susceptible*	18 (51.42)
			Intermediate	4 (11.42)
			Resistant	13 (37.14)
4	Ciprofloxacin (5µg)	CIP	*Susceptible*	2 (5.71)
			Intermediate	18 (51.42)
			Resistant	15 (42.85)
5	Levofloxacin (5 µg)	LEV	*Susceptible*	2 (5.71)
			Intermediate	19 (54.28)
			Resistant	14 (40)
6	Ampicillin (10µg)	AMP	*Susceptible*	2 (5.71)
			Intermediate	5 (14.28)
			Resistant	28 (80)

In heart the prevalence was 25 % (5/20), in kidney and liver the ratio of *Salmonella species* was 30 % (6/20) and 35 % (7/20) respectively.

In chicken leg piece the high prevalence (45 %) (9/20) was noticed, While In chicken chest piece the prevalence of *Salmonella species* was 40 % (8/20).

The prevalence of *Salmonella species* were more in Highway bypass and Kohat main city 44 % (11/25) and 40 % (10/25) repectively, in college town area of kohat the ratio of *Salmonella species* was 36 % (9/25), while the lowest prevalence (24 %) (6/25) of *Salmonella species* in poultry samples were observed in KDA.

Antibiotic susceptibility patterens

Most *Salmonella species*were resistant to Ampicillin (80%) and Tetracycline (77.14%) (Table 4 and Fig. 3). Out of 35 samples 14 (40%) were susceptible to Azithromysin, 11(31.42%) were intermediate and 10(28.57%) were resistant to Azithromysin. Chlorompenicol was found to be effective drug against *Salmonella species* isolates. In 35 samples 18 isolates (51.42%) were sensitive to Chlorompenicol, while 13(37.14%) were resistant to the current antibiotic and 4(11.12%) were intermediate sensitive to chlorompenicol.

The broad spectrum DNA syntheisis inhibitors Ciprofloxacin and Levofloxacin did not show effective

result against *Salmonella species* and most of them were intermediate sensitive (51.52%) and (54.28%) respectively. In current study, the Cholorompenicol was the most effective (51.42%) drug of against our isolates of *Salmonella species*.

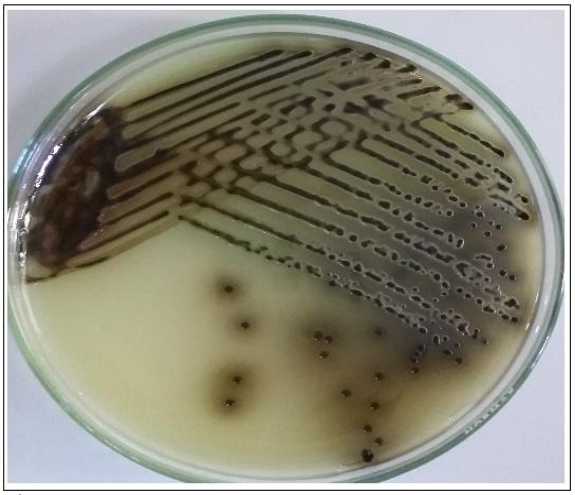

Fig. 1. Growth of *Salmonella species* on bismuth sulphite agar.

Discussion

Anthropozoonotic diseases include salmonellosis, one of the serious medical problems in food industry. For salmonella food poisoning in human poultry is a major source [S. Parveen, 2007]. In 2012, several outbreaks of *Salmonella* have been associated with poultry meat and products [CDC, 2010]. Poultry are the most important reservoir for *Salmonella,* with prevalence in chicken carcasses ranging from 20-70% in most countries [J. Y. D. Aoust, 1989].

The high prevalence of *salmonella* in chicken meat may be a result of cross-contamination from intestines during processing and cutting or from cages, floor and workers during retailing or marketing. In the present study the prevalence of *Salmonella species* based on Tetrathionate broth as enrichment media were 35%. These results showed a lower prevalence rate then the finding done by Shah and Korejo *et al.,* [A. H. Shah, 2012] which is 48.75% in Sindh, Tandojam, Pakistan and came compatible with findings done by previous studies Arroyo *et al*, [S. Arroyo, 2010] (31.4%). Our results are lower in prevalence than those obtained by Vera *et al.,* [J. G. Vera, 2007] which his result (58.6%) from chicken meat when used Tetrathionate broth as pre enrichment media 42 ˚C. In our study, a total of 80% of the *Salmonella* isolates were found to be resistant to Ampicillin but this was much lower than that reported in isolates from foods from Poland where 93% were Ampicillin-resistant [T. W. R Chia, 2009].

This high antimicrobial resistance might be due to extensive use or misuse of Penicillin. In our study, 100% (35/35) of the isolates were resistance to at least one of the antibiotic tested.

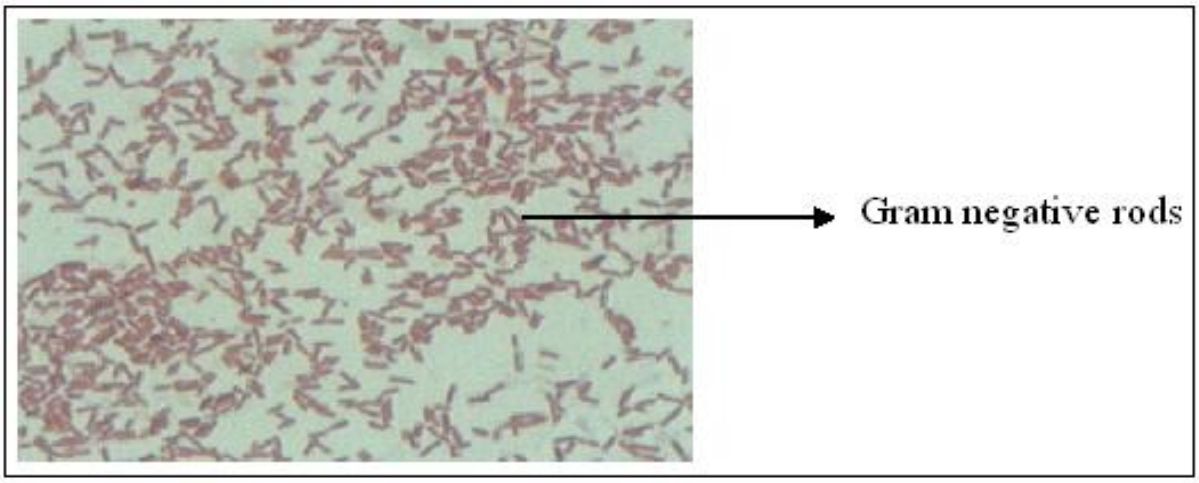

Fig. 2. Gram staining of *Salmonella species*.

The presence of multiple-resistant isolates in meat may cause serious human poisoning; the isolates may be transferred from meat to humans, not only by direct contact but also indirectly by consumption of meat or other food contaminated with *Salmonella* Depoorter *et al*, [P. Depoorter, 2012], because

antibiotic resistance genes frequently are located on mobile genetic elements. Horizontal gene transfer between food bacteria and human intestinal bacteria has been demonstrated, not only in vitro, but also in vivo [L. M. Glenn, 2011].High resistance to tetracycline (77.14%) was observed in our study. Tetracycline is used as a growth enhancer in poultry production.

This practice has greatly promoted the occurrence of antimicrobial-resistant isolates. In comparison to our study (77.14%), a higher incidence (100%) of resistance to Tetracycline was reported by Glenn *et al*, [D. A. Benson, 2008].Isolates showed resistance to two antibiotics from the quinolones class, namely Ciprofloxacin (42.85%) and Levofloxacin (40%). Comparatively low incidence of resistance to ciprofloxacin has also been observed in other studies. Only 3% of *Salmonella* isolated from the retail meats was resistance to ciprofloxacin according to a survey in Malaysia [S. Singh, 2012]. Outbreaks of quinolone-resistant *Salmonella* infections have been reported in the United States [D. Wasyl, 2012].

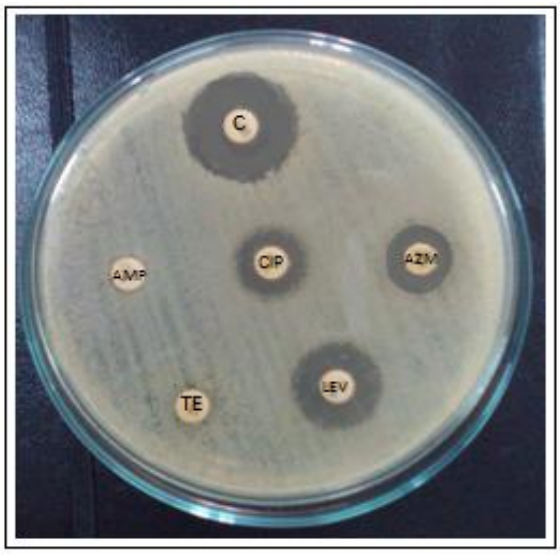

Fig. 3. Showing antibiotic susceptibility pattern of *Salmonella species*.

In our study, we found high incidences of resistance against chloramphenicol, a class of Amphenicols (37.14%),). Chloramphenicol is commonly used as veterinary medicines in livestock production. Recently, an increasing occurrence of resistance to this antibiotic has already been demonstrated in many countries [E. G. Iossifidou, 2012]. The appearance of antimicrobial resistant *Salmonella* isolates may result in serious human and livestock infections.

Conclusion

This study confirm the high prevalence (35%) of *Salmonella species* in the poultry samples and this study also confirm the high prevalence of antibiotic resistance of *Salmonella species* isolated from poultry samples of District Kohat.

The antibiotic resistance profile indicates the limited therapeutic value of different antibiotics including, Ampicillin, Tetracycline, Ciprofloxacin, Levofloxacin, Azithromycin and Chloramphenicol. There is a need for continued surveillance is emphasized to determine regular antimicrobial susceptibility data to identify the changing pattern of resistance. Keeping in view the several possibilities of *Salmonella* contamination in the poultry industry, specific epidemiological studies on the spread of *Salmonella* at various levels of production is needed on a long term basis.

References

Shah H, Korejo NA. 2012. Antimicrobial Resistance Profile of Salmonella Serovars Isolated from Chicken Meat. Journal of Veterinary and Animal Science **2,** 40-46.

Althouse CC, Patterson S, Fedorka-Cray P, Isaacson RE. 2003. Type 1 fimbriae of Salmonella enterica serovar Typhimurium bind to enterocytes and contribute to colonization of swine in vivo, Infectious Immunology **71,** 6446.

CLSI (Clinical and Laboratory Standards Institute). 2012. Performance for antimicrobial disk susceptibility tests", "approved standard, CLSI document M02-A11 ed.11th, Wayne (PA), USA: Clinical and Laboratory Standards Institute **32(1),** 1–76.

CDC, the Centers for Disease Control and Prevention. 2010. National Antimicrobial

Resistance Monitoring System for enteric bacteria (NARMS), "Human isolates final report, 2008", G. A. Atlanta, The Centers for Disease Control and Prevention.

Scherer CA, Miller SI. 2001. Molecular pathogenesis of Salmonella, "Principles of bacterial pathogenesis" (E. A. Groisman), Academic Press, United States of America 265-316.

Scherer CA, Miller SI. 2001. Molecular pathogenesis of Salmonella, Principles of bacterial pathogenesis (E. A. Groisman), Academic Press, United States of America, 265-316.

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. 2008. GenBank", Nucleic Acids Research **36,** 25-30.

Newell DG, Koopmans M, Verhoef L, Duizer Aidara E, Kane A, Sprong H, Giessen VD, Kruse HJ. 2010. Food-borne diseases-the challenges of 20 years ago still persist while new ones continue to emerge, International Journal of Food Microbiology **139,** S3-S15.

Benson DA, Karsch-Mizrachi I, Lipman DJ, Ostell J, Wheeler DL. 2008. GenBank, Nucleic Acids Research **36,** 25-30.

Wasyl D, Hoszowski A. 2012. First isolation of ESBL-producing Salmonella and emergence of multiresistant Salmonella Kentucky in turkey in Poland, Food Research International **45,** 958-961.

Iossifidou EG, Abrahim A, Soultos ND, Triantafillou EA, Koidis PA. 2012. Antimicrobial resistance profiles in Salmonella spp. and Escherichia coli isolates from turkey samples in Northern Greece, Annals Microbiology **62,** 623-628.

Duguid JP, Anderson ES, Campbell I. 1966 Fimbriae and adhesive properties in Salmonella, Journal of Pathology and Bacteriology **92,** 107.

Cox JM, Pavic A. 2010. Advances in enteropathogen control in poultry production", Journal of Applied Microbiology **108,** 745-755.

Aoust JYD. 1989. Salmonella, Food-Borne Bacterial Pathogens (M. P. Doyle), Marcel Dekkar, New York 327-445.

Vera JG, Fedorka-Cray PJ. 2007. Prevalence, distribution and characterization of ceftiofur resistance in Salmonella enterica isolated from poultry form in the USA from 1999 to 2003, International Journal of Antimicrobial Agents **30(2),** 134-42.

Todar K. 2005. Salmonella and salmonellosis, Todar Online Textbook of Bacteriology, Department Of Bacteriology, University of Wisconsin – Madison.

Glenn LM. Lindsey RL, Frank JF, Meinersmann RJ, Englen MD, Paula J. 2011. Analysis of antimicrobial resistance genes detected in multidrug resistant Salmonella enterica serovar Typhimurium isolated from food animals, Microbial Drug Resistance **17,** 407-418.

Thomson NR, Clayton DJ, Windhorst D, Vernikos G. 2008. Comparative genome analysis of Salmonella Enteritidis PT4 and Salmonella Gallinarum 287/91 provides insights into evolutionary and host adaptation pathways, Genome Research **18,** 1624.

Depoorter P, Persoons D, Uyttendaele M, Butaye P, De Zutter L, Dierick K. 2012. Assessment of human exposure to 3rd generation cephalosporin resistant Salmonella (CREC) through consumption of broiler meat in Belgium, International Journal of Food Microbiology **159,** 30-38.

Krieg RN, Holt JG. 1984. Bergey's manual of systemic Bacteriology" USA, Williams and Wilkins Company, Baltimore 308 – 429.

Parveen S, Taabodi M, Schwarz JG, Oscar TP, Harter-Dennis J, White DG. 2007. Prevalence and antimicrobial resistance of Salmonella recovered from processed poultry, Journal of Food Protection **70,** 2466-2472.

Arroyo S, Yadav AS, Singh SM, Bharti P. 2010. Prevalence of Salmonella in chicken eggs collected from poultry farms and marketing channels and their antimicrobial resistance, Food Research International **43,** 2027-2030.

Chia TWR, Goulter RM, McMeekin T, Dykes G, A, Fegan N. 2009. Attachment of different Salmonella serovars to materials commonly used in a poultry processing plant, Food Microbiology **26,** 853-859.

Porwollik S, McClell M. 2003. Lateral gene transfer in Salmonella, Microbial Infection **5,** 977.

Singh S, Agarwal RK, Tiwari SC, Singh H. 2012. Antibiotic resistance pattern among the Salmonella isolated from human, animal and meat in India", Tropical Animal Health and Production **44,** 665.

Lorian V. 1986. Antibiotics in laboratory medicine ed. 2nd, Williams & Wilkins, Baltimore.

Chuang Y, Wang CKC, Chen YT, Yang CH, Men SC, Fan CC, Chang LH, Yeh KS. 2008. Identification of the genetic determinants of Salmonellaenterica serotype Typhimurium that may regulate the expression of the type 1 fimbriae in response to solid agar and static broth culture conditions, BMC. Microbiology **8,** 126.