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Antibiogram of *Salmonella spp* Isolates from Raw Chicken Meat of Kathmandu Valley

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Abstract

Salmonella is one of the pathogenic microbe responsible in food borne diseases. In developing countries like Nepal, Salmonellosis is one of the leading food-borne disease. The present study was conducted with an objective to enumerate coliform and to find the prevalence of *Salmonella* species in chicken meat along with their antimicrobial susceptible profile. A total of 30 chicken meat samples were collected and examined following the standard techniques and procedures at the Med Micro Lab from January 2020 to April 2020. The study was performed following the conventional methods for the detection of *Salmonella* spp. Biochemical methods were implied for the detection of isolates and Antibiotic Susceptibility Test were performed by modified Kirby Bauer disc diffusion test [1]. Out of the 30 samples, 12(40%) sample showed positive for *Salmonella* spp. *Salmonella* spp 2(16.67%) were found to be resistant to Ciprofloxacin, Chloramphenicol 1 (0.33%), Cotrimoxazole 2(16.66%), Nalidixic acid 7 (58.33%) Ampicillin 3 (25%) and Ceftriaxone (0%). *Salmonella* was found to be 100% sensitive towards Ceftriaxone. The highest resistance was observed towards Nalidixic acid (58.33%) followed by Ampicillin (25%) and Cotrimoxazole (16.67%). Finally, the result of the study recommended that the use of standardized procedures in slaughtering and handling of chicken meat, provision of training on best practice of handling of meat for handlers and raising the level of awareness of people about the healthy consumption of chicken meat should be increased.

Keyword: *Salmonella* spps, Chicken meat, Antimicrobial Susceptibility test

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Introduction

Meat is an important source of protein and one of the most important food in the diet of the vast majority of people. Around 4.1 kg meat per person is consumed annually in context of Nepal (CBS, 2015). Meat is a suitable food for microorganisms to grow upon. Among all the micro-organisms, coliform and *Salmonella* are major cause of food borne human diseases like food poisoning, salmonellosis, typhoid, etc.[2]. Foodborne illnesses can be deadly, especially in young children. Diarrhoeal disorders are the most frequent illnesses caused by contaminated food, affecting 550 million people each year, including 220 million children under the age of five. Salmonella is one of the four major global causes of diarrhea. [3].

Food-borne disease are the main problem particularly in developing countries and cause the majority of illness and death around the world. Among these micro-organism coliform and *Salmonella* still a major cause of food borne human disease [4]. Salmonella is facultative anaerobes gram negative rod-shaped bacteria of the family Enterobacteriaceae. They are are non-spore-forming, primarily motile enterobacteria with peritrichous flagella and cell diameters around approximately 0.7 and 1.5 µm. [5].

Among the most prevalent food-borne diseases that pose a serious threat to global public health is salmonellosis. [6]. With the exception of elderly, small children, and individuals with impaired immune systems, this infectious disease has a self-limiting course and may not require antibiotic treatment. But it was noted that 3–10% of people with a gastrointestinal ailment brought on by Non-Typhoidal Salmonella are susceptible to bacteremia [7], a serious illness that could be fatal and needs to be treated with antibiotics, typically fluoroquinolones or extended-spectrum cephalosporins [8].

S. Typhimurium's antibiotic resistance rate has been rising in recent years, making it a global issue of growing concern that may result in detrimental effects on health. [9] . An antibiogram is a summary of the antimicrobial susceptibility testing outcomes of a particular microorganism to a range of antimicrobial medications [10]. In the case of awaited microbiological culture and susceptibility findings, the antibiogram aids the clinician and pharmacist in making the best immediate antimicrobial choice.

Antibiotic resistance is a global public health problem. Despite the fact that every country is affected by it, it remains unknown how severe the issue is in developing countries. [11]. Several fluoroquinolones and third-generation cephalosporins are the most frequently used antibiotics for treating salmonellosis in people. The earlier drugs Chloramphenicol, Ampicillin, Ciprofloxacin, Co-trimoxazole, Nalidixic acid, Ceftriaxone are occasionally used as alternatives [12]. S. Typhimurium outbreaks have been reported all over the world, and the majority of them came due to this organism's antibiotic resistance. [13]

Hence, This study was performed to enumerate coliform and examine Antibiotic Susceptibility Test (AST) of *Salmonella* isolates from raw chicken meat from different places in Kathmandu Valley. A prospective cross sectional study design was conducted in Med Micro Nepal Laboratory, Kathmandu from 2020 January – 2020 April to enumerate the coliforms and asses the prevalence of *Salmonella* spp and their Antibiogram testing from raw chicken meat sample.

Materials and methods

Sample size and sample site

A random sample of 30 retail grocery outlets, representing the valley were chosen and 30 raw samples were purchased over one week. The samples were collected from the most renowned shops of Kathmandu valley of Baneshwor, Bagbazzar, Sankhamul, etc.

Sample transportation

Chicken sample of 25 gram were collected and wrapped into Aluminium foil and placed into sterile polythene bag and transported to the laboratory within 1 hour of collection to minimize contamination. The samples were examined as soon as they reached the lab.

Selective enrichment

The meat sample was collected and minced with a sterile knife. Five gram of the meat sample was placed into a conical flask with 45 ml of selenite F broth and incubated for 24 hours at 37 °C [14].

Plating out and identification

After incubation for 24 hours, a loop full of inoculum from the selenite F broth was transferred and streaked on Xylose lysine deoxycholate agar (XLD)agar (HiMedia). The plates were incubated at 37°C for 24 hours and were examined for the growth of typical *Salmonella* colonies. After incubation, the plates were examined for suspected and typical colonies. If lactose fermentation occurs, the medium will turn transparent or translucent colorless due to the acidic pH. Pink colonies with or without black centers were produced by *Salmonella* spp. on XLD. *Salmonella, Shigella*, and other non-lactose fermenter appear as red or pink colonies. Colonies of *Salmonella* spp. appears with or without black centers (depending on the species isolated) as shown in **Photograph 1**.

Gram staining

The preliminary identification of microorganisms was carried out using Gram staining. Salmonella was detected as gram-negative rods using the standard approach.[15]

Biochemical confirmation

The isolated colonies from XLD were sub-cultured on nutrient broth (NB) and incubated at 37°C for 4 hours. From the nutrient broth inoculum was taken and streaked on Nutrient Agar (NA) and MacConkey agar (MA). The Petri-plates were incubated at 37°C for 24 hours. MA shows weather the organism is Lactose fermenter or Non lactose Fermenter. Thus, the isolated colonies from NA were subjected to different biochemical test[16]. The organism were identified primarily by catalase test, oxidase test, O/F test, IMViC, TSIA test. *Salmonella* sp. Shows the following biochemical indications: INDOLE (-ve), MR (+ve), VP (-ve), CITRATE (-ve), TSIA (Alkali/Acid, H2S +ve), UREASE (-ve), O/F (Fermentative) as shown in Photograph 2.

Antibiotic Susceptibility Test

According to Clinical and Laboratory Standard Institute Technique, all isolated organisms were tested for antibiotic susceptibility using the modified Kirby Bauer disc diffusion method on Mueller-Hinton Agar [16]. A sharply marginated circle of bacterial growth around the disk indicates the point at which bacterial growth outnumbers the inhibitory effects of the antimicrobial drug.The concentration of antimicrobial compound at this margin is known as the critical concentration, and it is roughly equal to the minimal inhibitory concentration obtained in broth dilution susceptibility testing.

For inoculums preparation, 3-4 pure culture were transferred into Nutrient Broth and incubated at 37°C for 2-4 hours to obtained turbidity to match 0.5% McFarland [17]. A sterile cotton swab was dipped into the inoculums, rotated pressing it against the upper inside wall of tube to remove the excess inoculums. Then carpet culture was done and it was allowed to dry for 10 minutes[18]. With the help of sterilized forceps, disc were carefully placed on the agar surface at least 15 mm away from the edge and pressed lightly to make

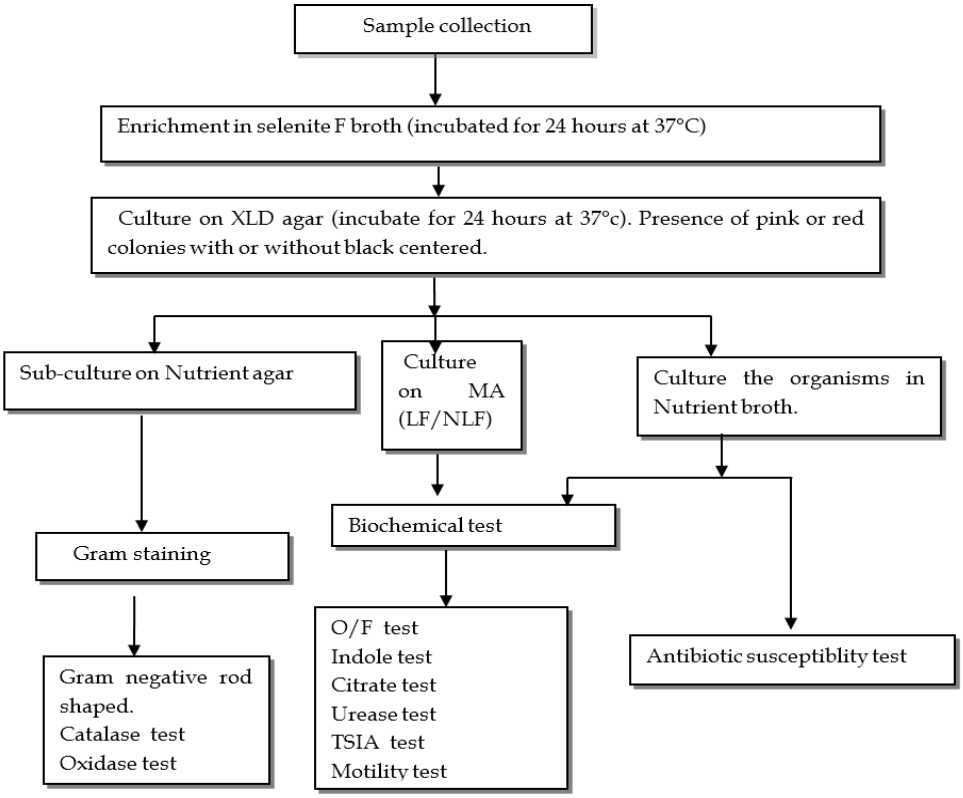


Figure 1. Flow chart showing procedure for isolation of *Salmonella* spp.

contact with the surface of the medium. The plates were left to incubate for 24 hours at 37 °C. The diameter of the inhibitory zone was measured following incubation [19].

Antibiotics used for susceptibility test were: Nalidixic acid (30 mcg), Co-trimoxazole (25 mcg), Ampicillin (10 mcg), Chloramphenicol (30 mcg), Ciprofloxacin (5 mcg), Ceftriaxone (30 mcg).

The CLSI publishes tables that list the antimicrobial drugs that are acceptable for testing members of the *Enterobacteriaceae, Pseudomonas,* and other gram-negative glucose nonfermenters, *Staphylococci, Enterococci, Streptococci, Haemophilus* species, and other gram-negative glucose nonfermenters. A standardized, threshold-based assessment scheme has been created for the goal of simplification, in which the degree of drug effectiveness is classified as "susceptible," "intermediate," or "resistant," depending on the MIC value.

Susceptible: A bacterial strain is considered to be susceptible to a certain antibiotic when it is suppressed in vitro by a concentration of this drug with a high possibility of therapeutic success.

Intermediate: A bacterial strain's sensitivity to a certain antibiotic is considered to be intermediate when it is suppressed in vitro by a concentration of this drug linked with an unknown therapeutic effect.

Resistant: A bacterial strain is considered to be resistant to an antibiotic when it is suppressed in vitro by a concentration of the drug associated with a high risk of therapeutic failure. [20]

Purity plate

The purity plate was used to check the purity of the inoculum used for the biochemical tests and to determine if aseptic conditions were maintained during the performance of the tests. The test organism was streaked in half portion of nutrient agar plate before inoculating in biochemical test tubes and the same inoculums of test organism was streaked on remaining half portion of nutrient agar plates. The plates were allowed to incubate for 24 hours at 37 °C. The development of some organisms in the pink form in the pre-and post-inoculum indicates that the environment has remained sterile[21].

Quality control

During the identification of the organism, one plate from each lot of created agar plates was placed in the incubator to assess their quality. Quality of sensitivity test was maintained by adjusting the thickness of Mueller-Hinton agar at 4 mm and pH at 6.9-7.0. The appropriate quantity of antibiotic discs was also utilized, as recommended. Aseptic practices were strictly followed throughout the entire procedure.

Data Analysis

The finding was statistically analyzed using Microsoft Excel v 2016.

Table 1. Inhibition Zone size interpretation chart (CLSI, 2015)

Antimicrobial agent used	Code	Disc content	Resistance	Intermediate	Sensitive
Chloramphenicol	C	30mcg	17	-	17
Ampicillin	AMP	10mcg	13	14-16	17
Co-trimoxazole	COT	25mcg	13	14-15	16
Nalidixic acid	NA	30mcg	13	14-18	19
Ciprofloxacin	CIP	5mcg	19	20-21	22
Ceftriaxone	CTR	30mcg	20	21-22	23

Disposal

Cultures plates, contaminated swabs were placed in the disposal bag and autoclaved prior to discard.

Table 2. Sample collected (number and type of meat collected)

S.N	Sample site	Growth in XLD agar
1.	Babarmahal	No
2.	Shrinagar	Yes
3.	Sahayoginagar	Yes
4.	Subidhanagar	No
5.	Sankhamul	No
6.	New baneshwor	Yes
7.	Thapagaun	Yes
8.	Bagbazar A	Yes
9.	Bagbazar B	Yes
10.	Bagbazar C	Yes
11.	Bagbazar D	Yes
12.	Gahanapokhari A	Yes
13.	Gahanapokhari B	No
14.	Kalopul A	Yes
15.	Kalopul B	Yes
16.	Greenland	No
17.	Basundhara	Yes
18.	Dhapasi	Yes
19.	Trilingtar	Yes
20.	Nature club	No
21.	Samakushi	Yes
22.	Raniban A	No
23.	Raniban B	No
24.	Raniban C	No
25.	Dhungedhara A	Yes
26.	Dhungedhara B	Yes
27.	Thulobharyang A	No
28.	Thulobharyang B	No
29.	Ratnapark	Yes
30.	Swyambhu	Yes

Results

Out of total 30 samples examined, 19(63.3%) showed positive growth on XLD agar. (**Table 2**)

Distribution of positive culture on XLD

Out of 30 samples, the growth of the organism was found in 19(63.33%) samples and no growth of the organism was found in 11(36.67%) samples. Among these 19 samples, 12 were *Salmonella* positive. (Figure 2)

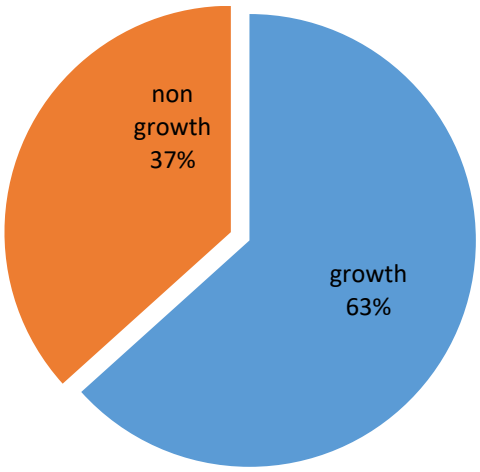


Figure 2: Pie-Chart showing distribution of positive culture on XLD

Antibiotics susceptibility patterns of bacterial isolates from chicken sample

The antibiotics susceptibility tests of isolates were carried out by using Co-trimoxazole, Nalidixic acid, Chloramphenicol, Ampicillin, Ciprofloxacin, Ceftriaxone. The result of the antibiotic susceptibility test represented below(**Table 3**).

Table: 3 Antibiotic susceptibility patterns of total *Salmonella* isolates

Antibiotics	Dose (mcg)	Sensitive		Intermediate		Resistance	
		No	%	No	%	No	%
Chloramphenicol	30	10	83.33	1	8.33	1	8.33
Ampicillin	10	9	75	0	0	3	25
Co-trimoxazole	25	10	83.33	0	0	2	16.67
Nalidixic acid	30	2	16.66	3	25	7	58.33
Ciprofloxacin	5	5	41.66	5	41.65	2	16.67
Ceftriaxone	30	12	100	0	0	0	0

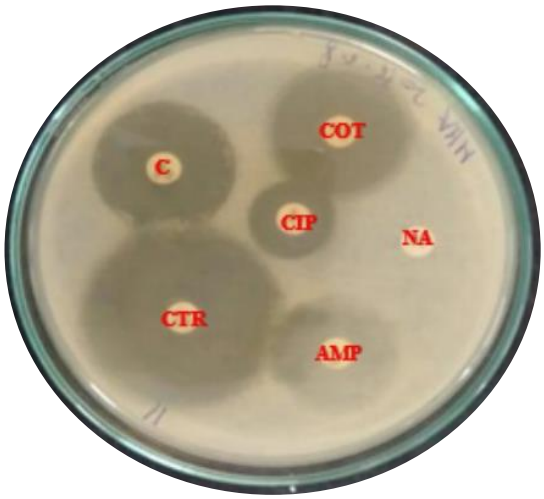
Index Numbers : Observable colony on plate

Discussion

Salmonella is present in nearly all animal groups, and eating contaminated food is typically a factor in human illness. Salmonellosis can also spread by direct contact with water, animals, and on occasion, humans.[22]

Photograph 1. Growth of *Salmonella* on XLD agar (Pink colonies with black centers)

Photograph 2. Biochemical test for *Salmonella* spp. From left to right: INDOLE (-ve), MR (+ve), VP (-ve), CITRATE (-ve), TSIA (Alkali/Acid, H2S +ve), UREASE (-ve), O/F (Fermentative)



Photograph 3. Antibiotic Susceptibility test of *Salmonella* spp. (Zone of inhibition shown by *Salmonella* sp. against Ceftriaxone{CTR}, Chloramphenicol{C}, Co-trimoxazole{COT}, Ciprofloxacin{CIP}, Ampicillin{AMP}, Nalidixic acid{NA})

Among the 30 samples, 12 spp. (40%) were found to be contaminated with Salmonella *spp*. In this study, 58.33% of the isolates were found to be resistant towards Nalidixic acid, followed by Ampicillin (25%), Ciprofloxacin (16.66%), Co-trimoxazole (16.67%), Chloramphenicol (8.33%), and Ceftriaxone (0%). Ceftriaxone (100%) was found to be the most sensitive towards salmonella followed by Chloramphenicol (83.33%) and Co-trimoxazole (83.33%). Chloramphenicol sensitivity was 98%.

Salmonellosis has to be treated and controlled with antimicrobial chemotherapy due to the failures of various approaches to prevent and control it in the food animal industry, including enhanced biosecurity, vaccination, the use of competitive exclusion products, and the introduction of novel immune potentiators [12]. Since the majority of *Salmonella* infections are contracted through the consumption of contaminated foods of animal origin, the use of antibiotics in food for animals is likely responsible for the increasing prevalence of *Salmonella* that is resistant to antibiotics [23].

Sharma et. al(2019) found Non-Typhoidal *Salmonella* highly prevalent on the poultry farms of Chitwan District Nepal. In his study, The farm level point prevalence rate was found to be 55% (10 of 18 farms) for *S. enterica.* [24]. Shafini et al (2017) compared chicken meat with beef meat and found chicken meat samples (40.4%) showed greater presence of *Salmonella* compared to beef (15.4%)[25]. In the study conducted by Margarita et al (2017), 40% (8/20) of the isolates were resistant towards Sulfamethoxazole, 25% (5/20) for Nalidixic acid, Ciprofloxacin, Ampicillin, and 20% (4/20) for Tetracycline. Ceftazidime, Cefotaxime, Meropenem, Azithromycin, and Tigecycline were all effective against all isolates [26]. Ali et al (2015) found *Salmonella enterica* resistant to four antibiotics including Ampicillin, Chloramphenicol, Tetracycline and nalidixic acid, which coincides with our study i.e. resistant to Nalidixic Acid [27].

Through genetic mutation and the acquisition of resistance-encoding genes, the use of antibiotics in animal food has led to the rise of antibiotic resistance. [13]. The development of Nalidixic-resistance is believed to be caused by genetic factors, such as mutations in the DNA gyrase (gyrA and gyrB) and topoisomersase IV (parC and pare) genes. [28]. In Enterobacteriaceae, two or more point mutations in the DNA gyrase and topoisomerase IV QRDRs usually result in complete fluoroquinolone resistance. [29].

There were several limitations to the present study. This is a community based cross-sectional study of the meat sample and its contaminant. This study was conducted from random groceries of Kathmandu. Hence, it doesn't necessarily reflect the picture of whole country. The isolate was tested against few perils of antibiotics. However, this small study shall contribute in an effective way in public health concerns. Through this study, a strong hygiene policy regarding meat distrubution can be made possible starting from local level. The main concern of antibiotic resistance of the *Salmonella* can be verified through clinical samples taken from actual patients taking this study as a reference. The verification will then further contribute in the appropriate drug selection against the infection caused by *Salmonella* isolates.

Thus, regular monitoring of meat shops and quality of meat is essential to prevent the spread of *Salmonella* spp. and health hazard caused by them. The findings are significant for amending local public health policy.

Conclusion

The present study focused on detection of *Salmonella* and assesing their antibiotic susceptibility test from 30 different meat samples of Kathmandu valley.

Out of the 30 meat samples, 12(40%) were positive for *Salmonella*. From this study, it was found that 16.67% of the isolates were resistant to Ciprofloxacin, 8.33% to Chloramphenicol, 16.67% to Co-trimoxazole, 58.33% to Nalidixic acid, 25% to Ampicillin. Both *Salmonella* Typhi and *S.* Paratyphi were found to be 100% sensitive towards Ceftriaxone which shows Ceftriaxone (100%) is the most effective antibiotics towards *Salmonella* spp.

Author's contribution

The development of the concept, preliminary work, and laboratory analysis, was done by AS, AA,

DO, PD, SKJ, and BG under the guidance of SS. The final manuscript was read and approved by every author.

Competing interests

The authors affirm that they have no competing interests.

Funding

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Ethical approval and consent

The relevant authorities provided their consent for this study to be performed.

Data availability

Upon request, the information will be made available.

References

1. Biemer JJ. Antimicrobial susceptibility testing by the Kirby-Bauer disc diffusion method. Annals of Clinical & Laboratory Science. 1973; 3(2):135–40.
2. Carramiñana JJ, Rota C, Agustin I, Herrera A. High prevalence of multiple resistance to antibiotics in Salmonella serovars isolated from a poultry slaughterhouse in Spain. Veterinary microbiology. 2004 Nov 30;104(1-2):133-9.
3. https://www.who.int/news-room/fact-sheets/detail/salmonella-(non-typhoidal)
4. CIDRAP (2006): Center for Infectious Disease Research and Policy, Academic Health Centre, University of Minnesota. http://www.cidrap.umn.edu/contents/fs/fooddisease/causes/salmoview.html (Accessed on 03/05/2007)
5. Fàbrega A, & Vila J. Salmonella enterica serovar Typhimurium skills to succeed in the host: virulence and regulation. Clinical microbiology reviews, 2013 26(2), 308–341. https://doi.org/10.1128/CMR.00066-12
6. Antunes P, Mourão J, Campos J, & Peixe L.. Salmonellosis: the role of poultry meat. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases, 2016; 22(2), 110–121. https://doi.org/10.1016/j.cmi.2015.12.004
7. Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien, Pablos-Mendez A, Klugman KP. Antimicrobial resistance in developing countries. Part I: Recent trends and current status. Lancet Infect Dis 2005; 5:481–493.
8. Hohmann EL. Nontyphoidal salmonellosis. Clin Infect Dis 2001;32:263–269.
9. Parisi A, Crump J, Glass K, Howden B, Furuya-Kanamori L, Vilkins S, et al. . Health outcomes from multidrug-resistant Salmonella infections in high-income countries: a systematic review and meta-analysis. Foodborne Pathogens and Disease. 2018; 15, 428–436. doi: 10.1089/fpd.2017.2403
10. https://www.health.state.mn.us/diseases/antibioticresistance/abx/antibiograms.pdf
11. Ang JY, Ezike E, Asmar BI Antibacterial resistance. Indian journal of Pediatrics 2004;71: 229- 239.
12. WHO (2005): WHO media centrehttp://www.who.int/mediacentre/factsheets/fs139/en/print.html (Accessed on 08/08/2007)
13. Martínez M, Retamal P, Rojas-Aedo J, Fernández J, Fernández A and Lapierre L. . Multidrug-Resistant Outbreak-Associated Salmonella strains in irrigation water from the metropolitan region, Chile. Zoonoses Public Health 2017; 64, 299–304. doi: 10.1111/zph.12311
14. Gorski L. . Selective enrichment media bias the types of *Salmonella* enterica strains isolated from mixed strain cultures and complex enrichment broths. PloS one, 2012; 7(4), e34722. https://doi.org/10.1371/journal.pone.0034722
15. Giannella RA. Salmonella. In: Baron S, editor. Medical Microbiology. 4th edition. Galveston (TX): University of Texas Medical Branch at Galveston; 1996. Chapter 21.
16. Holding AJ & Collee JG Chapter I Routine Biochemical Tests Methods in Microbiology Volume 6, Part A, 1971, Pages 1-32
17. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH, Eds. Manual of clinical microbiology. 7th ed. Washington, DC: ASM, 1999.
18. Akbar A, & Anal AK. . Isolation of Salmonella from ready-to-eat poultry meat and evaluation of its survival at low temperature, microwaving and simulated gastric fluids. Journal of food science and technology, 2015 52(5), 3051–3057. https://doi.org/10.1007/s13197-014-1354-2
19. Day JB, Basavanna U, & Sharma SK. Development of a cell culture method to isolate and enrich Salmonella enterica serotype enteritidis from shell eggs for subsequent detection by real-time PCR. Applied and environmental microbiology, 2009;75(16), 5321–5327.

20. Rodloff A, Bauer T, Ewig, S, Kujath P, & Müller E. . Susceptible, intermediate, and resistant - the intensity of antibiotic action. Deutsches Arzteblatt international, 2008; 105(39), 657–662.

21. CLSI, "Performance Standards for Antimicrobial Susceptibility Testing (26th ed.) CLSI M100-S20.," Clin. Lab. Stand. Inst., no. 27, p. 63, 2016, Accessed: Dec. 10, 2021.

22. Ethelberg S, Olsen KE, Gerner-Smidt P, Molbak K.. The significance of the number of submitted samples and patient-related factors for fecal bacterial diagnostics. Clinical Microbiology and Infection 2007;13:1095–1099.

23. Antunesa P, Réub C, Sousab JC, Peixeb L, Pestanab N. Incidence of Salmonella from poultry products and their susceptibility to antimicrobial agents. Int J Food Microbiol. 2003;82:97–103.

24. Sharma S, Fowler PD, Pant DK, Singh S, Wilkins MJ Prevalence of non-typhoidal Salmonella and risk factors on poultry farms in Chitwan, Nepal, Veterinary World, 2021;14(2): 426-436.

25. Shafini AB, Son R, Mahyudin NA, Rukayadi Y, Zainazor TT. Prevalence of Salmonella spp. in chicken and beef from retail outlets in Malaysia, International Food Research Journal 2017 ;24(1): 437-449

26. Margarita Terentjeva, Jeļena Avsejenko, Madara Streikiša, Andra Utināne, Kaspars Kovaļenko, Aivars Bērziņš, Prevalence and antimicrobial resistance of Salmonella in meat and meat products in Latvia Ann Agric Environ Med. 2017;24(2):317–321

27. Akbar A, & Anal AK. Isolation of Salmonella from ready-to-eat poultry meat and evaluation of its survival at low temperature, microwaving and simulated gastric fluids. Journal of food science and technology, 2015;52(5), 3051–3057. https://doi.org/10.1007/s13197-014-1354-2

28. Weigel LM. Anderson GJ, & Tenover FC. DNA gyrase and topoisomerase IV mutations associated with fluoroquinolone resistance in Proteus mirabilis. Antimicrobial agents and chemotherapy, 2002;46(8), 2582–2587.

29. Hirose K, Hashimoto A, Tamura K, Kawamura Y, Ezaki T, Sagara H, Watanabe H. DNA Sequence analysis of DNA gyrase and DNA topoisomerase IV quinolone resistance-determining regions of Salmonella enterica serovar Typhi and serovar Paratyphi A. Antimicrob Agents Chemother. 2002;46:3249–52