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Isolation, Identification, and Antibiotic Susceptibility Profiling of Salmonella spp. Isolated from Meat and Milk Source, Nineveh, Iraq.

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Abstract

Salmonella has a significant impact on global health. It is one of the most widespread diseases in the world, causing serious intestinal and serological illnesses, depending on its species. This study aimed to isolate and identify *Salmonella* from different food sources, namely red meat, chicken products, and milk leaches, and to study its susceptibility to antibiotics. Samples were collected from different areas and markets in Nineveh Governorate. *Salmonella spp.* were isolated using selective xylose lysine XLD and SS agars. Out of 180 samples, 66 were positive divided into three food groups, were identified as local food sources in red meat give-up (90%), (70%) and (80%) for minced Meat, cuts and sausage respectively, while chicken products reported (40%), (30%) and (40%), From its sections chicken meat, chicken eggs and chicken liver, respectively, and milk products which its percentages are (30%), (30%) and (40%) And belonging to Yogurt, Cheese and Cream respectively, in imported sample collected of this present study red meat give (40%), (20%) and (30%) for every section of Minced, Meat cut and sausage respectively, although chicken products scored (40%), (20%) and (20%) positive samples affiliated with chicken meat, chicken eggs and chicken liver respectively, and dairy was (10%) ,(20%) and (10%) results for Yogurt, Cheese and Cream respectively, Antibiotic susceptibility testing was also performed to determine the degree of its antibiotic resistance of the 66 positive samples which had identified from this food groups were amoxicillin (36.8%), (64.3%) and (42.4%), amoxicillin-clavulanate (52.6%), (64.3%) and (42.4%), cefotaxime (15.8%), (50%) and (45.5%), ceftazidime (89.5%), (85.7%) and (63.6%), cephalexin (42.1%), (57.1%) and (51.5%), meropenem (10.5%), (14.3%) and (24.2%), doxycycline (78.9%), (64.3%) and (51.5%), ciprofloxacin (5.3%), (14.3%) and(24.2%), norfloxacin (26.3%), (35.7%) and (24.2%), levofloxacin (5.3%), (14.3%) and (24.2%), Gentamicin (42.1%), (50%) and (45.5%), Streptomycin (78.9%), (78.6%) and (63.6%) and

Trimethoprim (63.1%), (78.6%) and (63.6%), all results which mentioned had resistance ratios of positive food groups samples were to 19 chicken products, 14 milk products and 33 red meat respectively.

Keywords: *Salmonella*, Food contamination, Antibiotic sensitivity, enteritis, Isolation

Introduction

Salmonella bacteria are a primary cause of foodborne illness in humans worldwide. They are zoonotic diseases capable of infecting both humans and animals. Their proliferation results in substantial global economic losses due to the wide range of maladies they cause, from basic gastrointestinal issues like diarrhoea to serious systemic infections like typhoid fever (Gast & Porter Jr, 2020). *Salmonella* infection is usually transmitted through the consumption of infected animals and contaminated meat, and it is always spread through the food chain from farm to table, especially through foods of animal origin such as chicken, its eggs and products, pork, and fish, as well as the consumption of raw meat and minced meat, which has high-value protein humans need (Giromini & Givens, 2022; Yada, 2023). It is possible for the meat of cattle known to be reservoir hosts for *Salmonella* to become contaminated with *Salmonella* during slaughter as well as during subsequent processing steps (Roels et al., 1997). *Salmonella* reaches the eggs through the hen's ovary, as eggs are the primary source of *Salmonella enteritidis* bacteria that cause food poisoning (salmonellosis) (Bäumler et al., 2000). *Salmonella* may thrive in chicken liver at temperatures encountered during secondary processing in a processing plant, restaurant, institution, or home kitchen because of its high nutritional content, pH, and water activity (Bovill et al., 2000). Since milk contains the essential nutrients the body needs in the right amounts, it has generally been referred to as a nearly perfect food. Nonetheless, it is a very effective way to expose a huge number of individuals to possible microbiological risks. Dairy product safety in relation to foodborne illnesses is a significant worldwide concern, particularly in underdeveloped nations where milk and milk products are produced using subpar agricultural, sanitary, and hygienic methods (Jordan, 2007). Dairy products play a major role in nutrition, people's health, and livelihoods worldwide. However, dairy products are not free from the risk of contamination by microorganisms affecting consumers, regardless of the traditional hygiene practices of developing countries, especially the heat treatment of milk (pasteurization) (de Klerk & Robinson, 2022). The advancements in human and animal health and well-being are largely due to antibiotics. However, a worrying increase in antibiotic resistance among bacterial groups has been observed recently. The continued resistance to these antibiotics leads to a buildup of health problems, especially when treatment options are limited (Wise, 2008). *Salmonella* is a bacterial disease that can be transmitted to humans and animals. One of the distinctive characteristics of this bacterium is that it has more than one host, which helps it to spread. Most animals and birds are natural reservoirs and carriers of it to humans, and this happens through contamination by food and the surrounding environment (Krämer et al., 2011; Matheson et al., 2010). The primary requirement of this study is to identify *Salmonella* bacteria after isolating them from local and imported food sources and then to determine the sensitivity of a range of antibiotics to the positive isolate

Materials and Methods

The samples for this study, which included red meat, chicken meat and liver, as well as dairy products, were collected between September 2025 and February 2026 in different areas from Nineveh Governorate, Iraq. The samples were equally divided between locally produced and imported products, totaling 160 samples of chicken products and their products, 50 of dairy products, and 20 of red meat. All samples were collected under excellent sterilization and proper storage conditions.

25 gm was taken from each sample. This quantity is crushed in 50 ml of distilled water. Then, 1 ml of this water is taken into a tube containing 4 ml of brain heart infusion broth (BHI) as enrichment media, which will be incubated at 37°C for 18-24 hours until culture is performed in special selective media (Angelo et al., 2015; Vibbert et al., 2015).

Use selective media for isolation and identification of *Salmonella spp.:* samples were cultured on selective media, including Salmonella-Shigella agar (S-S) and xylose lysine deoxycholate agar (XLD). The solidification of the media was checked to ensure sterility by incubating it at 37°C for 24 hours, after which it was refrigerated for later use (ATLAS et al., 2010). The agar will turn red due to the presence of Salmonella colonies, and a grey area of spread will not appear in negative samples.

Microscopic discrimination: Gram stain was used for the colonization and morphological differentiation of *Salmonella* bacteria, as well as the ability of this stain to differentiate between two main classes of bacteria.

***Salmonella spp*. sensitivity to antibiotic susceptibility testing:** The purpose of the sensitivity test is to determine which antibiotics are effective against a given bacterium and which are not. Typically, sensitivity tablets, known as Kirby-Bauer tablets, are used on Müller-Hinton agar after it has been incubated for 24 hours at 37°C. Bacterial samples are collected in a measured amount of saline solution (0.5 McFarland Turbidity Standard) after shaking the agar. A sterile cotton swab is used to press against the inner wall of the tube to remove excess liquid. The plate is then carefully marked more than three times, covering all areas of the agar, to ensure uniform culture. Using sterile forceps, 14 tablets of different antibiotics are placed on two plates, representing one of each different positive sample, resulting in isolation to be tested after light pressure is then applied to each tablet to ensure it is firmly attached.

Results and discussion

Results of red meat contamination in *Salmonella* spp.: The isolation and identification of *Salmonella spp.* in collected local and imported food samples (Table 1)

revealed 30 local red meat samples divided into 10 meat cuts, 10 minced, and 10 sausages. It has been represented by 7 (70%), 9 (90%), and 8 (80%) positive samples, respectively. Minced meat had the highest percentage (90%) in this local group and all isolates; then imported group samples were also minced meat with high contamination (40%) in 30 imported red meat samples, which has been divided into 10 meats cut, 10 minced, 10 sausages were resulted 2(20%),4(40%) and 3(30%) samples respectively**.**

Table 1: *Salmonella* spp. isolated from imported and local samples of red meat

Type of sample	No. of sample	Minced	Meat cut	sausage	No. and percentage of Total sample
Local	No. Total sample	10	10	10	30
	No. and percentage of Positive sample	9(90%)	7(70%)	8(80%)	24(80%)
Imported	No. Total samples	10	10	10	30
	No. and percentage of Positive samples	4(40%)	2(20%)	3(30%)	9 (30%)

In local red meat group samples, percentages that contained *Salmonella spp.* bacteria in the current study (80%) sausage, (70%) meat cut, (90%) minced. percentage results of sausage were higher than [15], (Abdel-Atty et al., 2023; Soares et al., 2021) in Karbala governorate, Iraq, Egypt and Brazil, who reported (4%), (0%) and (5%), respectively. Also, meat cut contaminated with salmonella in our study had a higher rate than each of the reports [18], [19], [20] in Ethiopia, Pakistan, and south Africa who reports (14.29%), (12%) and (1.25%) respectively. The *Salmonella-infected* percentage in minced red meat was the highest compared to other studies, such as (Ameen & Althwani, 2025; Khudair et al., 2022) in the Karbala and Baghdad governorates, Iraq, who reported (9%) and (45%), However, this study (Santos et al., 2020) in the Philippines has a rate of (86.67%), which is close to the positive samples in local minced meat. The reason for this high percentage in red meat, especially minced meat, is the use of tools that have not been washed properly, such as the mincer and knife, and the failure to wash hands thoroughly. Other contributing factors include mincing different parts of the cow's body, such as the tongue muscle and lymph nodes, which are more susceptible to bacteria, or parts from another infected cow. Additionally, the slaughtering area is not washed properly.

The group of imported red meat infected with salmonella in our study passed through the percentages of (30%) sausage, (20%) meat cut, and (40%) minced sausage, showing results of salmonella contamination higher than (Khudair et al., 2022; Müller et al., 2018) in countries Iraq and Germany who found (0%) and (1.1%) respectively. Meat cut results were lower, which was found in (Hawas & Al-Samarai, 2024)in the capital city in Iraq in percentage (34.7%), while it was higher than what was found (Sayed et al., 2021) in Assiut province, Egypt, who reported (2%), Also, *Salmonella* bacteria in minced meat was higher than in (Khudair et al., 2022) who reported (30%) in Iraq; the percentage was also higher than (Kusumaningrum & Dewanti-Hariyadi, 2012) who resulted in (12%) in Indonesia. Due to poor storage during transport and unloading, and because of cutting with contaminated machinery, the hands of the workers.

Results of chicken product contamination in *Salmonella spp*.: The results of the current study are presented in the (tablet-2) that 30 samples of local chicken products were divided into 10 chicken meats, 10 chicken eggs and 10 chicken livers and gave percentages of 4 (40%), 3 (30%) and 4 (40%) positive samples.

Respectively, chicken liver and meat were similar in proportion (40%) and were the highest in this local group of chicken meats. These samples were also presented in the imported chicken product sample group; chicken meats had the highest percentage of contamination (40%), which included 30 samples divided into 10 chicken meats, 10 chicken livers and 10 chicken eggs, producing 4 (40%), 2 (20%) and 2 (20%) positive samples, respectively.

Table 2: Salmonella spp. isolated from imported and local samples of chicken products

Type of sample	No. of sample	Chicken meat	Chicken eggs	Chicken liver	No. and percentage of Total sample
Local	No. Total sample	10	10	10	30
	No. and percentage of Positive sample	4(40%)	3(30%)	4(40%)	11(36.66%)
Imported	No. Total sample	10	10	10	30
	No. and percentage of positive sample	4(40%)	2(20%)	2(20%)	8 (24.00%)

The presentations of samples from the local chicken products group containing *salmonella spp* in this study (40%) chicken meat, (30%) chicken eggs and (40%) chicken liver showed salmonellosis infection in chicken meat were lower than (Sedeeq Ahmed et al., 2023) who reported (57%) in Egypt; however, it was close to (Yildirim et al., 2011a) who give percentage (44.1%) in Anatolia, turkey, in chicken egg the score was lower than (Rahman et al., 2019) who gave up (100%) in Bangladesh, while it was higher than (Soria et al., 2024) who scored (1.7%) in Argentina. Due to monitoring and thorough egg washing, the study showed that the local chicken liver was higher than (Ahmed & Khudor, 2019; Taib & Jakhsi, 2019) who reported (10%) and (4%) Basrah and Duhok, Iraq, respectively, and the reason is bacteria reaching the liver from other parts, such as the intestines, during slaughter and also poor storage.

Imported chicken products in the current study which have contamination in *salmonella spp.* are (40%) chicken meat, (20%) chicken eggs and (20%) chicken liver, chicken meat found higher than (Boshkoska et al., 2024; Mohammed et al., 2023) who scored (0%) and (17.5%) in Macedonia and Diyala Province, Iraq, while the results are equal to (Adzitey et al., 2025) in Ghana, who gave up

(40%) also, Imported chicken eggs are half of percentage (Al-Ruaby & Issa, 2014) who reported (40%) in Wasit governorate, Iraq, Although it is higher than (Khan et al., 2022) in Trinidad and Tobago by infection (0%), chicken liver in with *salmonella spp.* is higher than (Khudair et al., 2022) in Karbala, Iraq, by (8%), however it resulted lower than (Ahmed & Khudor, 2019) who reported (90%) in Basra, Iraq. The reason goes back to higher contamination. This is due to the use of unclean equipment, the transfer of bacteria from the intestines to other parts of the chicken, poor storage and transportation, unclean feed, contaminated water, Likewise, mixing the infected with the uninfected.

Results of milk products' contamination with *Salmonella spp.*: The third food main group, from which *Salmonella spp.* was isolated (Table-3), also contained local and imported food samples of dairy products collected in various areas of Nineveh Governorate, including 30 local samples presented in 10 yoghurts, 10 cheeses and 10 creams, which resulted in 3 (30%), 3 (30%) and 4 (40%) positive samples, respectively. Local cream had the higher contamination between them (40%). In contrast, among the imported samples of dairy products, cheese had the highest percentage (20%) of salmonella contamination in 30 imported samples divided into 10 yoghurts, 10 cheeses and 10 creams, resulting in 1 (10%), 2 (20%) and 1 (10%) positive sample, respectively.

Table 3: *Salmonella spp*. isolated from imported and local samples of milk products

Type of sample	No. of sample	Yogurt	Cheese	Cream	No. and percentage of Total Sample
Local	No.Total sample	10	10	10	30
	No. and percentage of positivesample	3(30%)	3(30%)	4(40%)	10(33.33%)
Imported	No.Total sample	10	10	10	30
	No. and percentage of positivesample	1(10%)	2(20%)	1(10%)	4 (13.33%)

In this study, samples from the local milk products group that had *salmonella spp*. in them were shown (30%) Yogurt, (30%) Cheese and (40%) Cream, *salmonella* which found in yogurt, is higher than (Alwan, 2024; Mahdi, 2019) who give up (0%) and (5%) respectively, in Baghdad, Iraq. Salmonellosis contamination in cheese was lower than (Habeeb, 2026) in Alkut province, Iraq, and higher than (Arif, 2012) who rescored (2.5%) Sulymania governorate, Iraq. Local cream results were higher than (Azeez & Makki, 2023) who reported (6%) in Almuthana governorate, Iraq.

While the imported milk products group which contain *salmonella spp* in current study reported (10%) Yogurt, (20%) Cheese and (10%) Cream, if yogurt is compared with another study yogurt is higher than (Alwan, 2024; Halim et al., 2022) by percentage (0%) and (3%) in Egypt and Baghdad, Iraq, respectively; *Salmonella* infection in cheese showed a higher percentage than (Temesgen et al., 2025) who give a percentage (0%) in central Ethiopia. one of the reasons for the high *Salmonella* spp. Contamination in this group of the current study is the use of poorly sterilized containers and utensils. Another important reason is the inadequate curing or pasteurization of milk to eliminate most microbes, especially bacteria. This is followed by poor storage and preservation during transport.

Xylose Lysine Deoxycholate (XLD) is also an exogenous medium in which *Salmonella* appears as small colonies that are red in color and also have black centers. As shown in the (Figure-1)

Salmonella can be isolated from samples using *Salmonella-Shigella* agar medium, which is a differential and selective medium between Shigella and *Salmonella* species. *Salmonella* bacteria grow on sodium thiosulfate and ferric citrate to enable the detection of hydrogen sulfide through the production of colonies with a black center. (Figure-2) SS agar plate.

Figure (1): The bacterial isolates of salmonella on XLD

Figure (2): The bacterial isolates of salmonella on SS agar

Under a light microscope, *Salmonella* appears as red rod-shaped colonies, as in the (figure-3) indicating that it is within the Gram-negative group after the Gram-Stain steps.

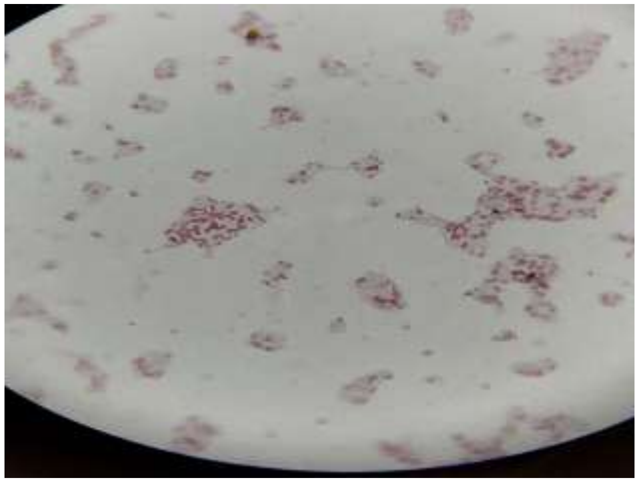

Figure (3): Show the gram-negative red rod-shaped colonies

The result of the antibiotic sensitivity test: The diameter of the area inhibited by the antibiotic discs was measured in millimeters in this study, which was compared to zone diameter interpretation standards from CLSI (2025). In general, the following antibiotics in this study exhibited the highest overall resistance rates among these three food groups by percentages: ceftazidime (75.7%), tobramycin (71.2%), streptomycin (66.6%), and doxycycline (62.1%). The results showed the positivity of three food group samples isolated from local and imported ones, presented by 66 samples. The 19 positive samples of chicken products exhibited the highest levels of resistance against antibiotics ceftazidime (89.5%), tobramycin (78.9%), doxycycline (78.9%), and streptomycin (63.1%). 14 positive samples of milk products appeared to have a higher resistance against ceftazidime (85.7%), tobramycin (78.6%), streptomycin (78.6%), and doxycycline (64.3%). While the 33 red meat positive group samples give up their resistances against ceftazidime (63.6%), tobramycin (63.6%), streptomycin (63.6%), and doxycycline (51.5%).

Conversely, isolated positive samples from the three food groups showed less resistance against antibiotics ciprofloxacin and levofloxacin in similar proportions are (5.3%), (14.3%) and (24.2%), against chicken products, milk products and red meat, respectively. After that, meropenem gave (10.5%), (14.3%) and (24.2%), then Cefotaxime (15.8%), (50%) and (45.5%) against chicken products, milk products and red meat, respectively. (table-4) appears with other results of other antibiotics.

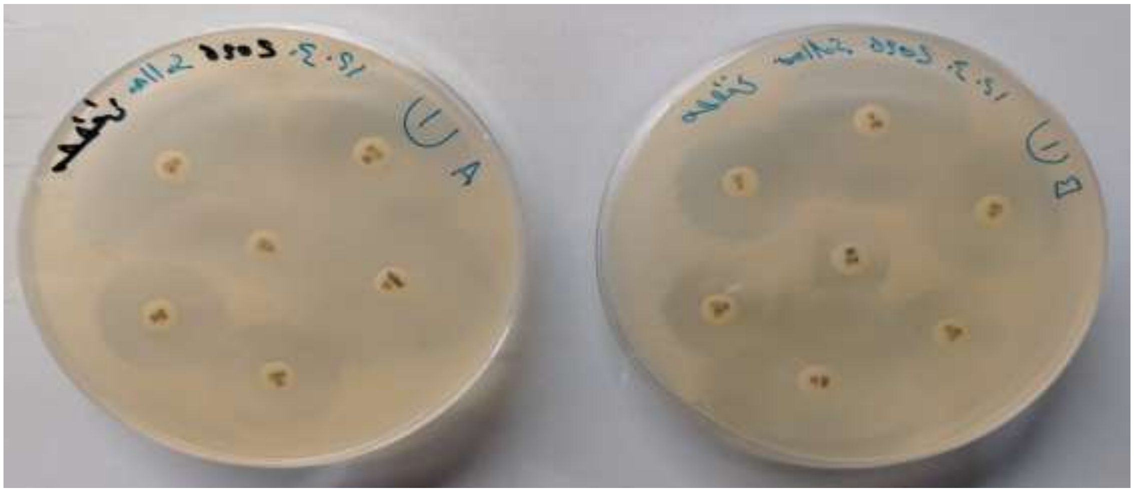

Figure (4): Antibiotic sensitivity test result for *Salmonella* spp.

In the present study, the first group of food that had been tested was milk products by antibiotic disc susceptibility. It was an antibiotic; ceftazidime has a higher percentage of resistance (89.5%) among others. Ceftazidime, if compared with other broiler studies, was higher than (Hassan & Alhatami, 2019; Punchihewage-Don et al., 2024; Tagar & Qambrani, 2023) who were reported as (0%), (2.5%), and (3.4%) in Pakistan, A Alnajaf governorate Iraq, USA, respectively, while resistance of cefotaxime in our study (15.8%) was lower than (Tagar & Qambrani, 2023; Yildirim et al., 2011b),who give (32.1%) and (86.7) in Pakistan and Turkey, respectively. gentamycin shows higher resistance than (Punchihewage-Don et al., 2024) who reported as (11%) and is a little higher than (Hassan & Alhatami, 2019) who resulted (37.5%); however, this antibiotic's resistance is less than (Rahman et al., 2019) by percentage (60%). High level of resistance to the mentioned antibiotics Due to prolonged and inappropriate use in poultry production and care.

In milk derivatives, ceftazidime also has the highest resistance (85.7%), and if compared with another study, such as (Atabey et al., 2021), it was the highest during the time that ciprofloxacin (14.3%) had a higher resistance than (Gebeyehu et al., 2022) in Ethiopia. Although it has less resistance than (Temesgen et al., 2025) Ethiopia, again, on the other side, gentamicin is equal to (Gebeyehu et al., 2022) in rate (42.5%) in both studies.

Red meat and meat products have antibiotic resistance, such as ceftazidime (63.6%), which has a high resistance percentage in this group of food and is also higher than (Fayanju et al., 2024) in Nigeria, who scored (41%), however it has less than (Abdel-Atty et al., 2023) who give (100%), Meropenem was comfortable with (Fayanju et al., 2024) in (25%) but it has a lower ratio of resistance than (Abdel-Atty et al., 2023) by (33.3%) proportion. Trimethoprim (36.4%) additionally has a higher rate than (Adzitey et al., 2025); in addition, amoxicillin-clavulanate resistance (42.4%) is also lower than in study (Abdel-Atty et al., 2023) in Al-Fayoum city, Egypt. Improper use has led to the spread of resistance to these antibiotics. This is also due to the use of antibiotics in cosmetics and biopesticides, poor hygiene, excessive use of these substances, and the presence of antibiotic residues in food and animal waste, as well as improper disposal. All of this contributes to the natural development of resistance genes (Endale et al., 2023).

Table (4): Antibiotic susceptibility test of Salmonella spp. isolates for the three food groups toward antibiotics

antibiotics / concentration	Chicken products No. (19)	Milk derivatives No. (14)	Red meat (beef) No. (33)	Total Resistance
AM (25 µg)	7 (36.8%)	9 (64.3%)	14 (42.4%)	(45.4%)
AMC (20/10µg)	10 (52.6%)	9 (64.3%)	14 (42.4%)	(50%)
CTX (30 µg)	3 (15.8%)	7 (50%)	15 (45.5%)	(37.8%)
CAZ (30 µg)	17 (89.5%)	12 (85.7%)	21 (63.6%)	(75.7%)
CX (30 µg)	8 (42.1%)	8 (57.1%)	17 (51.5%)	(50%)
MRP (10 µg)	2 (10.5%)	2 (14.3%)	8 (24.2%)	(18.1%)
D (10 µg)	15 (78.9%)	9 (64.3%)	17 (51.5%)	(62.1%)
CIP (10 µg)	1 (5.3%)	2 (14.3%)	8 (24.2%)	(16.6%)
NX (10 µg)	5 (26.3%)	5 (35.7%)	8 (24.2%)	(27.2%)
LE (5 µg)	1 (5.3%)	2 (14.3%)	8 (24.2%)	(16.6%)
GN (10 µg)	8 (42.1%)	7 (50%)	15 (45.5%)	(45.4%)
TOB (10 µg)	15 (78.9%)	11 (78.6%)	21 (63.6%)	(71.2%)
S (30 µg)	12 (63.1%)	11 (78.6%)	21 (63.6%)	(66.6%)
TMP (10 µg)	10 (52.6%)	8 (57.1%)	12 (36.4%)	(45.4%)

Conclusion

Daily food consumption is a major cause of *salmonella* transmission to and from humans and animals, whether locally produced or imported. The infection rate was highest in red meat (33%), in poultry products (28.7%), and in dairy products (21.2%) due to poor storage during transport and unloading and because of using contaminated machinery; likewise, the contaminated hands of the workers and other reasons. In addition, there is the problem of the emergence of antibiotic resistance, and this is one of the most serious problems facing humanity after the discovery of antibiotics, as some of the antibiotics tested in the study showed high resistance from these bacteria, such as ceftazidime, tobramycin, doxycycline, and others. Improper use has led to the spread of resistance to these antibiotics. This is also due to the use of antibiotics in cosmetics and biopesticides and poor hygiene.

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