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Multiple Drug Resistance *Salmonella* and Antibiotic Residues in Egyptian Animal Products

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ABSTRACT

Food of animal origin is considered a primary source of foodborne diseases. The misuse of antibiotics to treat and control many bacterial diseases in farm animals has led to multiple antibiotic-resistant pathogens in contaminated food that can seriously threaten public health. The present study aimed to highlight the impact of antimicrobial misuse in Egyptian beef meat, poultry, and dairy farms on the emergence of multiple antibiotic resistance *Salmonella* and the detection of antibiotic residues in milk. A total of 1050 samples were collected randomly from poultry (liver, intestinal content, and bone marrow), meat, and milk products from different Egyptian governorates. *Salmonellae* were isolated from the collected samples and subjected to antimicrobial sensitivity testing through disk diffusion test using the most commonly used seven antibiotics in veterinary fields (cefradine, ciprofloxacin, oxytetracycline, erythromycin, amoxicillin, ampicillin, and streptomycin). The detection of oxytetracycline residue in milk samples was performed by high-performance liquid chromatography (HPLC). Most isolated *Salmonella*e were multiple drug resistant with an incidence rate of 8.6%, 15.4%, and 4% from poultry, meat-associated products, and milk-associated products, respectively, from different governorates. Antibiogram test showed that the isolated *Salmonella* from poultry, meat, and milk samples were resistant to oxytetracycline at 100%, 31.4%, and 43%, to amoxicillin at 73.3%, 31%, and 50%, and to ampicillin 66.6%, 50%, and 57%, respectively. No resistance to ciprofloxacin was detected in *Salmonella* isolates from all samples. Using HPLC, oxytetracycline residues were detected in milk samples. In conclusion, more attention should be paid to the connection between the widespread emergence of antibiotic-resistant *Salmonella* in Egypt and the overuse of antimicrobials in poultry, dairy, and meat farms. This connection affects consumer health and increases the likelihood of resistance genes spreading between different bacterial species.

Keywords: Antibiogram, High-performance liquid chromatography, Multiple drug resistance, *Salmonella*

INTRODUCTION

Food poisoning caused by bacterial infection is a serious public health hazard worldwide, and most countries invest significant resources to combat it. *Salmonella* is one of the most prevalent food poisoning causes in Europe (Callejón et al., 2015; Myintzaw et al., 2020), leading to 91,857 human illnesses in Europe in 2018 (ECDC, 2020).

Salmonellosis is usually associated with consuming food products contaminated with *Salmonella*, particularly poultry, meat, and egg products. Contamination can occur through various means, including poor hand washing or contact with infected pets (Munck et al., 2020). Food handlers of "meat processing" and "ready-to-eat" foods are critical in the spread of *Salmonella* (Ehuwa et al., 2021). The marketing of improper food items and products produced under poor quality measures are considered the primary sources of the disease spread (FAO/WHO, 2004; Bettridge et al., 2014).

Multidrug resistance refers to bacteria's ability to withstand several types of antibiotics (three or more classes) that are structurally distinct and target different microorganisms (Nikaido, 2009). Antibiotic resistance (AR) is usually caused by antibiotic overuse, through which more bacterial species may acquire AR due to increased antibiotic use (Gelband et al., 2015). Misuse of antibiotics can result in bacterial resistance, increasing the burden of infectious diseases and healthcare costs (Nhung et al., 2017). Direct interaction with animals, exposure to animal waste, ingestion of raw meat, and contact with meat surfaces are common ways of transmitting resistant bacteria to humans (Marshall and Levy, 2011).

Farmers have resorted to the indiscriminate use of antibiotics as prophylactic and growth enhancement to fulfill the rising demand for poultry meat and eggs, which are the most important source of animal protein (Adesokan et al., 2015). This has resulted in antimicrobial-resistant strains of various pathogens, including *Salmonella* (Musawa et al., 2021). Different food animal species received antibacterial drugs, mostly in poultry. Animal production methods in Africa employ many antibiotics, including tetracycline, aminoglycosides, and penicillin. Therefore, the existing high levels of MDR and AR on the continent are worsened (Kimera et al., 2020).

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Antimicrobial tolerance is a growing concern in animal and human Salmonellosis (Su et al., 2004). *Salmonella* strains resistant to antibiotics are common in most parts of the world and have increased sharply in the last decade (WHO, 2018). Transmission from animal to human is rapidly becoming more frequent. The issue of antimicrobial drug resistance is more problematic in developing countries (Shrestha et al., 2017). Multidrug resistance is most commonly seen in *Salmonella* strains obtained from food (Gargano et al., 2021), especially poultry and poultry products (Ehuwa et al., 2021; Raji et al., 2021). The ability of these bacteria to transfer their resistance genes to a human bacterial pathogen is one of the significant concerns (Musawa et al., 2021).

Alarming multidrug resistance of the *Salmonella* serovars isolated from chicken embryos in Henan province, China, calls for an immediate reduction in the usage of antimicrobial medicines in chicken hatcheries. Additionally, various patterns among the Salmonella serovars by pulsed-field gel electrophoresis indicate the presence of several contamination sources (Xu et al., 2021).

Allergies, sensitization, and development of MDR bacteria are the most common human risks occurred by eating foods of animal origin containing antibiotic residues (Donoghue, 2003). Antibiotic residues can cause various severe health problems, including antimicrobial tolerance, immunopathological effects, autoimmunity, carcinogenicity, mutagenicity, nephropathy, hepatotoxicity, reproductive abnormalities, bone marrow toxicity, or allergy (Nisha, 2008). It is critical in food safety programs to monitor veterinary drug residues in raw animal products, such as milk, eggs, and meat, to ensure public health preventative measures (Botsoglou and Fletouris, 2001; FAO, 2015).

High-performance liquid chromatography (HPLC) is one of the most effective analytical chemical instruments, capable of distinguishing, recognizing, and quantifying antibiotics found in food, its use in the field of residual analysis is growing by the day due to the diversity of mobile stages, the availability of a wide range of column packings, and the variety of operating modes (Kebede et al., 2014). Several studies using HPLC technology for the characterization and identification of veterinary pharmaceuticals in premixes and medicated feeds are documented (Krasucka et al., 2010; Han et al., 2020). The present study aimed to detect MDR *Salmonella* from foods of animal origin as well as the detection of antibiotic residues in milk samples.

MATERIALS AND METHODS

Ethical approval

The current study was conducted on animal specimens, and no invasive procedures were performed on animals. This study did not involve any *in vivo* experiments. Poultry, milk and milk products, meat and meat by product samples were collected from the market for microbiological studies

Samples collection

A total number of 1050 poultry, milk and milk products, meat, and meat by-product samples were collected randomly from seven Egyptian governorate markets, including Giza, Cairo, Bani Suef, Fayoum, Alexandria, Menofia, and Qalyubia. These samples were divided as shown in Table 1.

Table 1. Source, type, and number of poultry, meat, and milk samples obtained from Egyptian markets

Source of samples	Type of samples	Number of examined samples
Poultry	Liver, intestinal content, and bone marrow	350
Meat and meat by-products	Beef meat, minced meat and liver, luncheon, sausage, hotdog, and kofta	350
Milk and milk by-products	Raw cow's milk, yogurt, and Kareesh cheese	350
Total	--	**1050**

Isolation and identification of *Salmonella*

All samples were collected aseptically and placed in separate sterile plastic bags, and transferred to the laboratory as soon as possible under hygienic conditions in an ice box. The samples were screened for isolation of *Salmonella* spp. The incubated samples were streaked over the surface of the SS agar medium (Oxoid) and incubated for 24 hours at 37°C before being analyzed for colony characteristics and cellular morphology. All bacterial isolates were identified by traditional methods, including morphological, biochemical, and colonial characters, according to Cruickshank et al. (1973) and Quinn et al. (2011), and confirmed by a test kit (BioMérieux, France).

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Antibiogram assay

The antimicrobial sensitivity tests were performed on confirmed *salmonella* strains using the disk diffusion technique according to Fine gold and Martin (1982) and the clinical laboratory standards institute (CLSI, 2020). Antibiotics used in the study included cefradine, ciprofloxacin, oxytetracycline, erythromycin, amoxicillin, ampicillin, and streptomycin. All antibiotic disks were procured from HI media laboratories (Mumbai, India).

High-performance liquid chromatography

Seven raw cow milk samples (15 ml) were randomly collected for testing oxytetracycline residues using the HPLC technique (Abbasi et al., 2011).

Analysis of analytical standards of oxytetracycline in milk samples

Solvents, reagents, and certified standards

Analytical standards of oxytetracycline, HPLC grade acetonitrile, methanol, and oxalic acid were obtained (Sigma Aldrich Company, USA), and the solid phase extraction (SPE) column (Bond Elut C18, 500 mg, 6 ml, Varian) were used for detection of oxytetracycline in milk. The water used for HPLC analysis was purified through Milli-Q water generated by a Milli-Q Plus Water Purification System (Millipore, USA).

Extraction of oxytetracycline from milk samples

Milk samples were prepared according to the methods by Abbasi et al. (2011). In brief, in a 50 ml plastic centrifuge bottle, a 15 ml milk sample was homogenized and combined with 25 ml Mcllvaine Buffer (Mixed citrate/phosphate, pH 4.1 with EDTA). The solution was vortexed for 1 minute before being centrifuged (Germany) at 10000 × g for 12 minutes at 4°C. The floating lipid layer and precipitate were removed, and the residual supernatant was extracted using SPE cartridges. The SPE cartridge was conditioned with 3 ml of methanol at a flow rate of 3 ml/minute before being washed with 2 ml of deionized water. At a flow rate of 5 ml/minute, the prepared mixture (centrifuged sample solution) was put into the SPE cartridge. The cartridge was rinsed with 1.5 milliliters of 5% methanol in deionized water. Elution was carried out at a rate of 4 ml/minute with 2 ml of HPLC-grade methanol. Drying samples by lyophilizing and reconstituting with 1 ml mobile phase, and 50 µl of the sample was injected into the HPLC column.

Chromatographic condition

High-performance liquid chromatography Agilent Series 1200 quaternary gradient pump, Series 1200 autosampler, and Series 1200 fluorescence detector with excitation and emission wavelengths were 255 and 365 nm, respectively. The HPLC software used was HPLC 2D Chemstation software (Hewlett-Packard, Les Ulis, France). The analytical column (stationary phase) was a reversed-phase C18 (25×04.6 mm, 5 µm) Teknorama (Spain). The mobile phase was a mixture of methanol, acetonitrile, and 50 mM oxalic acid (10: 20: 70% V/V). The mobile phase was mixed and filtered through a 0.45 µ filter (Nalgene, USA) and sonicated for 5 minutes to degas. The flow rate was 0.8 mL/minute. The retention time was 7.8 minutes.

Statistical analysis

The data were analyzed using IBM SPSS 25 program. The independent variables were tested for significance using the chi-square test, and the variables were found to be significant at $p < 0.05$.

RESULTS

Isolation and identification of *Salmonella* isolates

The suspected isolates were motile, Gram-negative, non-sporulated, and bacilli. It was a non-lactose fermenter on MacConkey agar medium while appeared as a colorless colony with a black center on SS agar media, and red colonies with black center colonies on Xylose lysine deoxycholate (XLD) agar medium. The suspected isolates were unable to ferment lactose, oxidase, indole production, Voges Proskauer, and urea hydrolysis were negative. Meanwhile, catalase and methyl red tests were positive and confirmed using a test kit (BioMérieux, France). Regarding the incidence rate of *Salmonella* spp. from different animal sources, the recovery rate of *Salmonella* spp. from poultry, meat and meat products, and milk and milk products were 8.6%, 15.4%, and 4%, respectively, from different governorates (Table 2).

Antibiotic sensitivity test of *Salmonella* isolated from different sources

The results of the antibiotic sensitivity test for *Salmonella* isolated from different sources are shown in Table 3 and Graph 1. *Salmonella* isolates from poultry samples revealed a high incidence of multiple AR. The highest resistance was found against oxytetracycline at 100% followed by amoxicillin at 73.3% and ampicillin at 66.6%. *Salmonella* isolates from meat and meat by-products showed a high incidence of multiple AR. The highest resistance was against cefradine

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at 53.7%, followed by ampicillin at 50%, then oxytetracycline and amoxicillin at 31.4%. Nearly the same results of the high incidence of MDR *Salmonella* were obtained from *Salmonella* isolates recovered from milk and milk by-products. The highest resistance incidence was found against erythromycin at 100%, followed by ampicillin at 57%, then cefradine and amoxicillin at 50%. All observed Chi-square values were higher than the expected value of the Chi-square test at 12 degrees of freedom. Thus, there was a strong relationship between the antibiotic and sensitivity.

Quantification of oxytetracycline concentration in milk samples

As shown in Table 5, the calibration curves of peak area relative to oxytetracycline concentration were plotted using data from 7 concentrations (0.05-5 g/ml milk). Using this technique, the standard curve was shown to be linear (R2 > 0.9995). The level of detection and limit of quantification for oxytetracycline were 0.0167 and 0.05 g/ml, respectively. Figures 1, 2, 3, and Table 4 provide the HPLC chromatogram for validation and standardization of oxytetracycline residues, the standard, blank milk sample, and positive sample with the standard, respectively.

Table 2. Incidence of *Salmonella* from different sources

Governorate	Poultry			Beef meat and meat by-products			Cow's milk and milk by-products		
	Percentage	+ve	Total	Percentage	+ve	Total	Percentage	+ve	Total
Cairo	50	5	10	50	8	16	50	1	2
Giza	50	4	8	50	6	12	50	2	4
Fayoum	50	6	12	50	9	18	50	2	4
Bany suif	50	3	6	50	7	14	50	2	4
Menofia	50	6	12	50	8	16	50	3	6
Alexandria	50	2	4	50	5	10	50	1	2
Qalyubia	50	4	8	50	11	22	50	3	6
Total	350	30	8.6	350	54	15.4	350	14	4
p value	0.753			0.731			0.911		
Test statistics	3.427			3.591			2.083		

Milk and meat were obtained from Cow, +ve: Positive for *Salmonella* isolation

Table 3. Antibiotic sensitivity test of *Salmonella* isolated from different sources of veterinary products in Egypt

Antibiotics	Poultry (30 isolates)						Meat and meat by-products (54 isolates)						Milk and milk by-product (14 isolates)					
	Resistance		Sensitive		Intermediate		Resistance		Sensitive		Intermediate		Resistance		Sensitive		Intermediate	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Cefradine	18	60	10	33.3	2	6	29	53.7	25	46.3	0	0	7	50	5	35.7	2	14
Ciprofloxacin	0	0	18	60	12	40	0	0	54	100	0	0	0	0	13	93	1	7
Oxytetracycline	30	100	0	0	0	0	17	31.4	33	61.1	4	7.4	6	43	7	50	1	7
Erythromycin	19	63.3	2	6	9	30	13	24	4	7.4	37	68.5	14	100	0	0	0	0
Amoxicillin	22	73.3	3	10	5	16.6	17	31.4	30	55	7	12.9	7	50	7	50	0	0
Ampicillin	20	66.6	8	26.6	2	6.6	27	50	25	46.3	2	3.7	8	57	4	28.5	2	14
Streptomycin	3	10	17	56.6	10	33.3	7	12.9	33	61	14	25.9	6	42.8	7	50	1	7
p value	49.04						200.4						101.26					

No: Number; %: percent

Table 4. The concentrations of Oxytetracycline concentration spiking in blank milk (µg/ml) and their corresponding peak response automatically using by high-performance liquid chromatography with recovery

Level	Amount of oxytetracycline in standard (µg/ml)	Area under peak	Concentration (µg/ml)	Recovery (%)
1	0.05	7.2	0.055981	111.96
2	0.1	15.75	0.117272	117.27
3	0.25	35.48	0.258705	103.48
4	0.5	73.179	0.528949	105.78
5	1	135.62	0.976555	97.65
6	2.5	337.45	2.423365	96.93459498
7	5	702.4	5.039494	100.7898781

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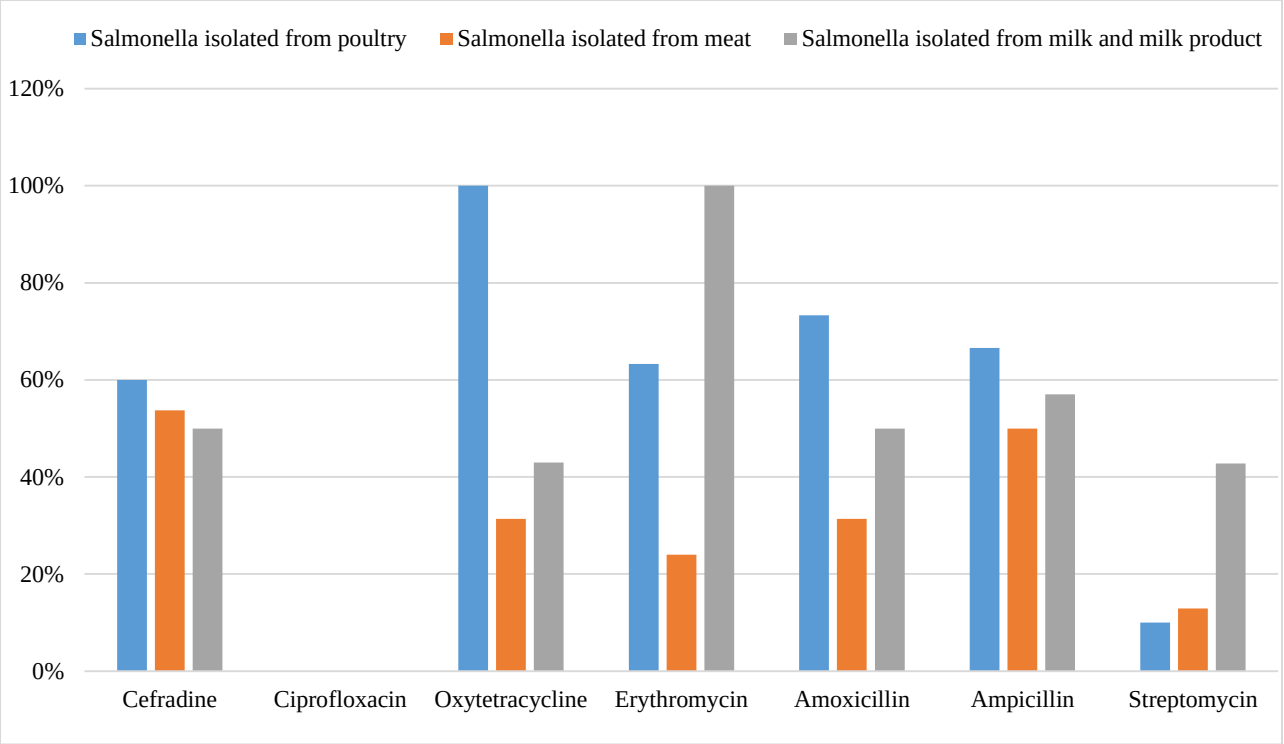

Graph 1. Antibiotic resistance of *Salmonella* isolated from different types of veterinary products in Egypt

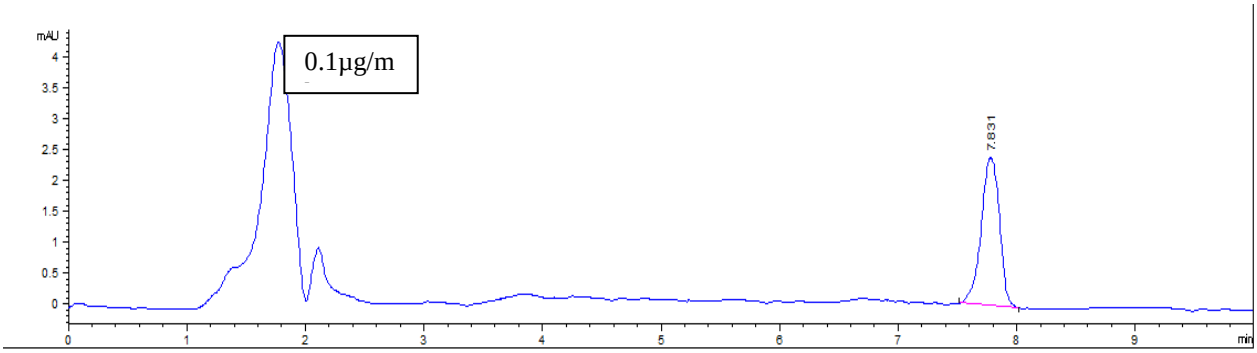

Figure 1. Cow milk from local Egyptian markets with oxytetracycline at a concentration of 0.1 µg/ml

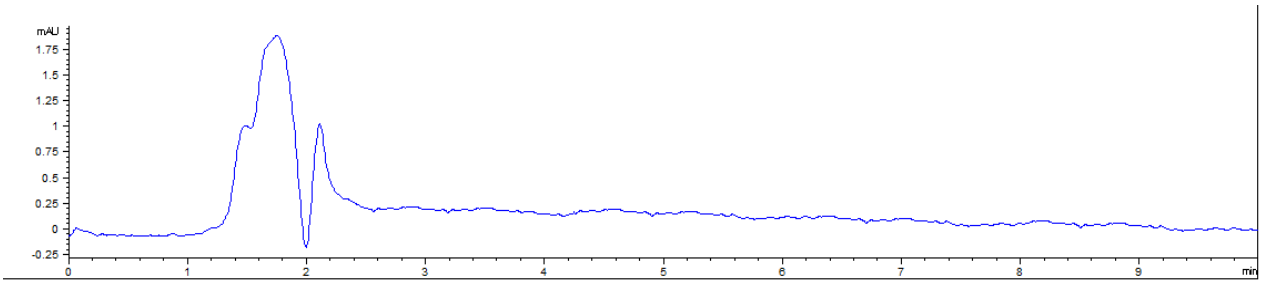

Figure 2. Blank cow milk sample from local Egyptian markets

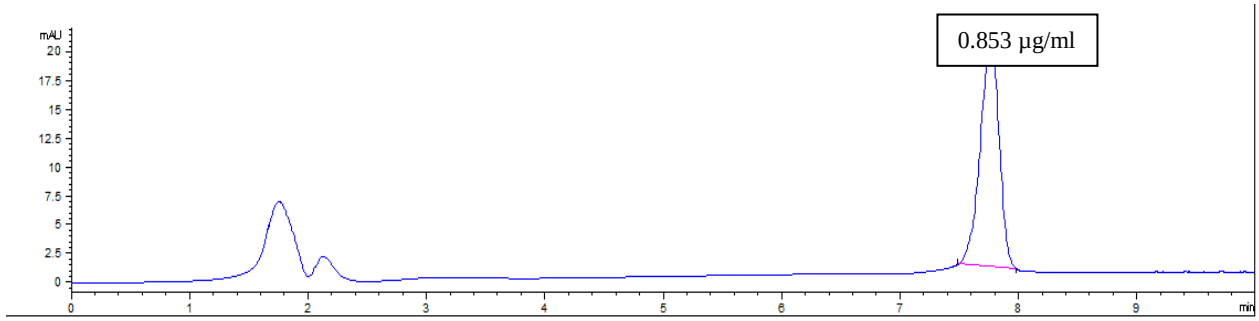

Figure 3. Cow milk sample from local Egyptian markets at a concentration of 0.853 µg/ml of oxytetracycline

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DISCUSSION

The use of antimicrobials in animals, particularly food-producing animals, has serious implications for both human and animal health since it can lead to bacterial resistance. The antibiotic-resistant bacteria (with resistance genes) developed in animals can be transmitted to humans by food intake, direct contact with food-producing animals, or environmental dissemination. Therefore, this section discusses the effect of antimicrobial use in Egyptian poultry, dairy and meat farms on the emergence of multiple AR *Salmonella*, and the detection of antibiotic residue in milk.

The results of the present study showed that the recovery rate of *Salmonella* spp. from poultry was 8.6% in different Egyptian governorates (Table 2). Raji et al. (2021) recorded nearly the same recovery rate as an overall *Salmonella* prevalence rate. However, higher results were reported by Endris et al. (2013), as the cultural prevalence of Salmonella among seropositive chicken was 35.7%. Fajilade et al. (2021) found that the incidence rate of *Salmonell*a in chickens was up to 100% in Ado-Ekiti, South Western Nigeria. In addition, the prevalence of Salmonellosis was 16.1% in poultry farms of Hawassa, Ethiopia (Endris et al., 2013), and it was 14.6%, as reported in Ethiopia by Ali et al. (2020). Ibrahim et al. (2014) indicated that the incidence of *Salmonellae* among local chicks was 21.67%, compared to 11.67% among imported chicks. Snow et al. (2007) found a rate of 10.7% for *Salmonella* recovered from poultry in the United Kingdom, and Ibrahim et al. (2013) isolated *Salmonell*a from broiler chickens at a rate of 16.66% in Beni-Suef governorate, Egypt.

The present results showed that the *Salmonella* incidence rate in meat and meat products was 15.4%. This result nearly agreed with Barrel (1982), who found a *salmonella* incidence rate of 17.6% for sausage, and Mrema et al. (2006), who reported a *Salmonella* prevalence rate of 20% for meat. In contrast to Abu Elnaga et al. (2021), who found that zero recovery rate of *Salmonellae* from raw meat of cows, goats, and sheep gathered from various retail marketplaces in Egypt. While a lower incidence rate was recorded by Abd El-Tawab et al. (2015), the obtained results indicated that the incidence of *Salmonell*a *enteritidis* in the examined samples of minced meat, sausage, and beef burger were 1/70 (1.4%), 1/40 (2.5%), and 0/40 (0%), respectively. Also, a high incidence rate of *Salmonell*a was reported by Fajilade et al. (2021) in Ado-Ekiti, South Western Nigeria, indicating recovered *Salmonella* from pork meat (100%), meat pie (71%), and Gala sausage (14%). Malkawi (2003) found a significant *Salmonella* prevalence of 81% for the studied minced meat.

The present results revealed that the recovery rate of *Salmonella* from milk and milk by-products was 4%. In the same line, Kunadu et al. (2018) found that MDR *Salmonella enterica* serovars Muenster and Legon were recovered as 11.8% and 5.9%, respectively, from unfermented cheese samples. While a higher prevalence was observed by Yasmin et al. (2015), their inspection divulged the presence of microorganisms with the harmful multidrug-resistant *Salmonella* spp. in 9 out of 35 samples from milk and the milk-based product collected from Dhaka metropolis, Bangladesh.

Elafify et al. (2019) found nine *Salmonella* isolates were recovered from raw milk (4/9, 44.4%) and Kariesh cheese (5/9, 55.5%), respectively. The antibiotic sensitivity testing showed that all isolates were resistant to Erythromycin and Streptomycin. Elafify et al. (2022) found that the highest *Salmonella* incidence rate was recovered from Kariesh cheese (16.67%), followed by market raw milk (6.66%), and soft white cheese (3.33%). In addition, Fajilade et al. (2021) observed a high prevalence of Salmonella in yogurt from Ado-Ekiti, South Western Nigeria (92%). Mhone et al. (2012) reported negative isolation of *Salmonella* spp. from processed and raw milk samples.

The cause of positive *Salmonella* isolation in food includes contamination due to infected people, environmental contamination (soil, vegetation, water) as well as animal food products, such as meat meal, bone meal eggs, or fish (Kariuki et al., 2006; Corry et al., 2002). Milk contamination may occur due to the fecal material of subclinically infected cows (Radke et al., 2002). *Salmonella* in milk poses a lower risk to public health through proper hygienic measures and pasteurization (Bankole et al., 2011).

This discrepancy in results might be attributed to differences in sample procedures, geographic location, and technique used. In Egypt, the most common serotype varies depending on where you live. This might be caused to contamination during the manufacturing, handling, packing, and storage of the product (Rabie et al., 2012).

Results of antibiotic sensitivity test of *Salmonella* isolated from different sources, *Salmonella* from poultry sources revealed a high incidence of multiple AR, the highest incidence of *Salmonella* resistance was against oxytetracycline 100% followed by amoxicillin 73.3% then ampicillin 66.6%, MDR isolates from poultry were found by Shrestha et al. (2017). Agada et al. (2014) announced that *Salmonella* recovered from poultry in Jos, Nigeria, was resistant to oxytetracycline (63%), ceftazidime (84%), and ampicillin (96%). The current study showed resistance to amoxicillin (73.3%), while that of Raj et al. (2021) was (100%).

While no ciprofloxacin resistance was detected, Raj et al. (2021) found ciprofloxacin resistance to *salmonella* isolated was 100% from the intestinal contents of slaughtered chickens and ready-to-eat chicken gizzards in Ilorin, Kwara State, Nigeria. Agada et al. (2014) reported that *Salmonella* isolated from poultry (poultry droppings, feeds, feces, and hand swabs from poultry farm workers and swabs from surfaces of intact eggshells) showed sensitivity to ciprofloxacin (81.6%) in Jos, Nigeria. However, Fashae et al. (2010) recorded a 3% resistance against ciprofloxacin in Ibadan, Nigeria. The isolated strains of the present study showed resistance to streptomycin (10%) in poultry samples,

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while Adesiji et al. (2011) revealed that the examined *Salmonella* isolates from pork meat samples were sensitive to ciprofloxacin and tetracycline by 100% but all were resistant to amoxicillin.

Mion et al. (2016) reported the sensitivity of *Salmonella* isolates isolated from poultry processing plants against ciprofloxacin by 94% and ampicillin by 77%, respectively. The current study showed resistance against ampicillin and erythromycin in 66.6% and 63.6%, respectively, in contrast to 100% resistivity of ampicillin and erythromycin in Dorgham et al. (2019). Ogu et al. (2021) found that *Salmonella* isolates from raw chicken meat were sensitive against gentamycin (40.39%) and resistant against ampicillin (96.15%). The multidrug-resistant *Salmonella* strains found in the study by Xu et al. (2020) in Henan, China, could pose a major threat to human and animal health. Thus, in order to prevent the spread of resistance to current antimicrobial agents, it is essential to monitor, regulate, and optimize the use of antimicrobial agents in chicken farms.

The results of antibiotic sensitivity of bacterial isolates from meat and meat by-products revealed a high incidence of multiple AR found between *Salmonella* isolates. The highest incidence of *Salmonella* resistance was against cefradine at 53.7%, followed by ampicillin at 50%, then oxytetracycline and amoxicillin at 31.4%. The antibiogram sensitivity test of *Salmonella enteritidis* isolates by Abd El-Tawab et al. (2015) indicated resistance to oxytetracycline and sensitivity to chloramphenicol, amoxicillin, levofloxacin, ciprofloxacin, enrofloxacin, and gentamycin, while Mezali and Hamdi (2012) recorded the resistance of 56 (90.32%) *Salmonella* isolates against at least one antibiotic, of which 20 isolates (32.26%) were MDR. The overall number of collected samples was 314 (128 raw chicken meat and poultry products, 144 raw red meat and meat products, and 42 processed meat products) from different market outlets. The *Salmonella* recovery rate was 61 (19.43%). The most significant recovery rate was recorded for red meat (23.61%, n = 34) and poultry (raw poultry meat and poultry products, 17.97%, n = 23).

The antibiotic sensitivity testing of *salmonella* isolates from milk and milk products in the current study revealed the highest incidence of resistance against erythromycin (100%), followed by ampicillin (57%), and then cefradine and amoxicillin (50%). Kunadu et al. (2018) found resistance to ciprofloxacin (100%), unlike the present results that were not resistant. Yasmin et al. (2015) found that 100% of *Salmonella* isolates showed resistance against ampicillin and ciprofloxacin.

Elafify et al. (2019) found that all *Salmonella* isolates from dairy products were resistant to erythromycin and streptomycin. In addition, Elafify et al. (2022) reported that all *Salmonella* spp. isolated from retailed dairies in Egypt, were resistant to oxacillin and nalidixic acid.

The results of AR of *Salmonella* isolates from all samples in the current study revealed a high resistance incidence of most *Salmonella* isolates from foods of animal origin. In the present study, erythromycin resistance from milk samples (100%) and poultry samples (63%) was recorded. High incidence of AR was recorded in milk samples (43% against oxytetracycline, 57% against ampicillin, and 50% against cefradin), poultry samples (100% against oxytetracycline, 66.6% against ampicillin, and 60% against cefradin), and meat samples (31.4% against oxytetracycline, 50% against ampicillin, and 53.7% against cefradin). It has been suggested that the increasing incidence of AR of Salmonellosis may result from misusing these antibiotics in veterinary fields (Economou and Gousia, 2015).

Rakitin et al. (2022) investigated the incidence of antimicrobial resistance among *salmonella enterica* strains obtained from food; their findings indicated that MDR against routinely used antibiotics as most of the strains (68.75%) exhibited multiple AR against the most commonly used antibiotics. The rise of MDR *Salmonella* strains is a worldwide public health concern. In Bangkok, Thailand, antimicrobial-resistant and virulent bacteria were obtained from retail food samples using the disc diffusion technique. It was found that the isolates were susceptible to amikacin and carbapenems. More than 30% of the isolates were resistant to ciprofloxacin, ampicillin, and tetracycline. Twenty isolates were resistant to at least three antimicrobial classes. Minimum inhibitory concentration revealed that about 12.07% of the isolates β-Lactam were resistant (Kong-Ngoen et al., 2022).

In the present study, using HPLC determination was one of the most used antibiotics in the field of oxytetracycline residues, six milk samples out of 7 (85.7%) contained residues exceeding the permissible limit (100 ug/l). The concentrations of oxytetracycline in seven samples were found to be 0.055 ug/ml, 0.011 µg/ml, 0.258 µg/ml, 0.528 µg/ml, 0.976 µg/ml, 2.423 µg/ml and 5.639 µg/ml. Abbasi et al. (2011) The mean of total tetracycline residues in all samples (114 samples) was 97.6 ± 16.9 ng/g, and that of pasteurized, sterilized, and raw milk samples were 87.1 ± 17.7, 112.0 ± 57.3, and 154.0 ± 66.3 ng/g, respectively. It was reported that the incidence of samples containing antibiotic residues was 25.4% of the total samples and 24.4%, 30%, and 28.6% of the sterilized, pasteurized, and raw milk samples, respectively. Al-Mazeedi et al. (2010) mentioned that more than 18% of milk samples had tetracyclines residues above the permissible limit in Kuwait. Navratilova et al. (2009) analyzed raw cow milk in Czech Republic and discovered tetracycline residues in 100% and oxytetracycline residues in 50.6% of tested samples. Ghimpeteanu et al. (2022) declared that all available food groups evaluated in different studies, including meat and meat products, milk and dairy products, eggs, honey, and non-animal-origin commodities, exhibit evidence of antibiotic residues.

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CONCLUSION

Misuse of antibiotics for treatment and control of bacterial infection in veterinary farms may give rise to multiple drug resistance *Salmonella* as well as increasing antibiotic residues in foods of animal origin which can be a major threat to public health. Health and veterinary authorities should prevent using any antibiotics on veterinary farms without the direct supervision of veterinarians and apply a strict application of suitable withdrawal period for different antibiotics before consuming food of animal origin. Therefore, periodic surveillance is strongly to find microorganisms with various forms of antibiotic resistance, advised.

DECLARATIONS

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Author's contribution

Ayman Ameen Samy designed the study and critically revised the manuscript. Amany Ahmed Arafa collected samples and performed bacterial isolation and biochemical typing. Riham Hassan Hedia participated in isolation, antibiotic sensitivity, and writing. Eman Shafeek Ibrahim took part in isolation, sensitivity, and revising the manuscript. All authors checked and approved the final version of the manuscript for publishing in the present journal.

Competing interests

There are no stated conflicts of interest by the authors.

Ethical considerations

All of the authors have reviewed the manuscripts for ethical concerns, such as plagiarism, consent to publish, misconduct, data fabrication and/or falsification, double publishing and/or submission, and redundancy.

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