

**Prevalence and Antimicrobial Sensitivity of Some Foodborne Pathogens
Isolated From Chicken Meat in Menoufia Governorate, Egypt**

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ABSTRACT

In Egypt, chicken meat is a stable protein source. It is highly susceptible to contamination by foodborne pathogens largely due to inadequate hygienic practice during processing, improper refrigeration, and poor handling in local markets. This study aimed to investigate the prevalence and antimicrobial resistance (AMR) of *Salmonella*, *Escherichia coli*, and *Staphylococcus aureus* in chicken breast, thigh, and liver samples (50 of each) collected from Menoufia Governorate, Egypt. The obtained results showed a high contamination rate of *Salmonella* (23.3%), *E. coli* (24%), and *S. aureus* (2%). Liver samples showed the highest prevalence of *Salmonella* (28%) and *E. coli* (30%), while *Staph. aureus* absent in liver samples. Serotyping identified *S. Typhimurium* (25.7% in liver), *E. coli* O127:H6 (13.8% in breast), and *S. aureus* enterotoxins SEA/SEB as dominant strains. Alarming, *Salmonella* exhibited 100% resistance to amoxicillin, apramycin, erythromycin, and cephradine, while *E. coli* showed universal resistance to amoxicillin and streptomycin. *S. aureus* isolates were fully resistant to erythromycin. These findings underscore significant public health risks due to multidrug-resistant pathogens in poultry products, necessitating urgent interventions to enhance food safety and regulate antibiotic use in Egypt's poultry sector.

Keywords: Chicken meat, *Salmonella*, *Escherichia coli*, *Staphylococcus aureus*, Antibiotic resistance.

1. INTRODUCTION

Chicken meat serves as an affordable and nutritious alternative to red meat in Egypt, providing high-quality protein, essential minerals, and vitamins (El Bayomi et al., 2018). However, it is highly susceptible to contamination by pathogens due to the nutrients that support microbial growth (Morshdy et

al., 2021). Contamination can occur during preparation, processing, distribution, and storage (Darwish et al., 2018). As a result, chicken products are often linked to foodborne pathogens like *Staphylococcus aureus*, *E. coli*, and *Salmonella*, which pose significant public health risks and contribute to zoonotic transmission, leading to widespread illnesses and

economic challenges globally (Abebe et al., 2020).

Salmonella is a significant foodborne zoonotic bacterium posing a global public health threat (Gomes et al., 2022), regarding animal-based foods like chicken, meat, and eggs being major sources of human salmonellosis. Non-typhoidal salmonellosis, caused by consuming *Salmonella*-contaminated foods, leads to vomiting, nausea, fever, diarrhea, and abdominal cramps within 12 to 36 hours of ingestion (Sams, 2001). Studies have frequently identified *Salmonella* spp. in chicken meat in countries like Vietnam, Japan, and Egypt (Luu et al., 2006; Iwabuchi et al., 2011; Darwish et al., 2018). Poultry meat and eggs serve as major reservoirs for *Salmonella* transmission through the food chain, contributing to human infections worldwide (Park et al., 2014).

Escherichia coli (*E. coli*) is a widely present bacterium that naturally resides in the intestinal tracts of humans, poultry, and other animals. While most strains are harmless and part of the normal gut flora, certain virulent strains have acquired genetic traits that enable them to cause diseases both within the intestines and in other parts of the body across various species. Diarrheagenic *E. coli* strains are particularly notable for causing enteric infections, with their pathogenicity determined by specific virulence factors that define six primary pathotypes. Among these, Shiga toxin-producing *E. coli* (STEC) stands out due to its strong association with severe illnesses in humans (Xia et al., 2010).

Staphylococcus aureus is a highly pathogenic bacterium that inhabits surfaces and mucous membranes, capable of causing various environmental and foodborne illnesses.

It is a leading cause of staphylococcal food contamination, which is economically significant and induces gastrointestinal symptoms such as vomiting and diarrhea within 2 to 6 hours of consuming contaminated food (Le et al., 2021). These effects are primarily caused by staphylococcal enterotoxins. Studies have identified *S. aureus* and its toxins in chicken meat and giblets sold in markets across Egypt, U.S., and Cambodia, highlighting its role as a major public health concern in the food chain (Darwish et al., 2018; Neyaz et al., 2020; Rortana et al., 2021).

The excessive use of antibiotics, particularly for non-therapeutic purposes such as promoting growth in broiler chickens and preventing diseases in poultry farming, has played a significant role in the development of antimicrobial resistance (Skočková et al., 2015). This widespread and often uncontrolled application of antimicrobials has led to the emergence of drug-resistant foodborne pathogens, posing a serious global public health threat (Alsayeqh et al., 2021).

The study aimed to assess the prevalence of foodborne pathogens in raw chicken meat and liver by isolating, identifying, and serotyping as *Salmonella* and *E. coli* species, as well as isolating and identifying *Staphylococcus aureus* and its enterotoxins. Additionally, evaluate the antimicrobial sensitivity of the isolated strains.

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Samples collection

A total of 150 fresh chicken samples, comprising breast, thigh, and liver sample (50 of each), were randomly collected from various locations in Menoufia Governorate, Egypt. The

samples were transported under aseptic conditions in sterile, labeled plastic bags and kept in a cool ice box to maintain their integrity during transit to the Food Hygiene Laboratory at Sadat University. Microbiological assessments were conducted to isolate and identify *Staphylococcus aureus*, *E. coli*, and *Salmonella*.

2.2. Methods

2.2.1. Samples preparation according to (ISO, 6887-2/2017)

A 25-gram of chicken meat sample was placed into a sterile blender jar containing 225 ml of 0.1% sterile buffered peptone water. The mixture was homogenized at 2000 rpm for 1-2 minutes to produce a homogenate. This homogenate was then used to prepare tenth fold serial dilutions, which were subsequently analyzed through various microbiological tests to detect and study pathogens.

2.2.2. Salmonella isolation and identification according to ISO (6579/2002)

Initially, the examined sample in buffered peptone water was incubated at 37°C for 18 hours to allow bacterial recovery. Next, 1 ml of the enriched sample was transferred to Rappaport Vassiliadis broth and incubated at 41.5°C for 24 hours to selectively promote *Salmonella* growth. Finally, a loopful of the enriched broth was streaked onto Xylose Lysine Deoxycholate (XLD) agar and incubated at 37°C for 24 hours. Suspected *Salmonella* colonies, appearing red with or without black centers, then identified according to Cruickshank et al. (1975).

2.2.2.1. The serological identification of Salmonella

It was performed following the Kauffman–White scheme (Kauffman,

1974), which is used to determine somatic (O) and flagellar (H) antigens. This process utilized *Salmonella* antiserum obtained from DENKA SEIKEN Co., Japan.

2.2.3. Escherichia coli isolation and identification according to ISO, (16649-2/2001)

Initially, one ml of the homogenate was placed in inverted Durham tubes with MacConkey broth and incubated at 37°C for 24 hours. Positive tubes were further enriched by transferring 1 ml into another MacConkey broth tube, which was incubated at 44°C for 24 hours. A loopful from the positive broth was streaked onto Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 hours. Suspected *E. coli* colonies appeared as metallic green, and purified colonies were transferred into nutrient agar slopes for confirmation. Isolated *E. coli* was biochemically identified according to MacFaddin (2000).

2.2.3.1. Serological Identification of Enteropathogenic E. coli

Using Rapid Diagnostic Antisera Sets (DENKA SEIKEN Co., Japan), according to Kok et al. (1996).

2.2.4. Staph. aureus isolation and identification according to FDA (2001)

A 0.1 ml aliquot of the prepared dilution was spread onto Baird-Parker agar plates, which were incubated at 35-37°C for 24-48 hours. During incubation, *Staphylococcus aureus* colonies appeared as black, shiny colonies surrounded by a clear zone. Suspected colonies were then biochemically identified following the methods described by Quinn et al. (2002).

2.2.4.1. Typing and detection of enterotoxins

As described by Shingaki et al. (1981), involved using kits designed to identify enterotoxins A, B, C, and D. The pure culture supernatant was subjected to serological test employing the Reverse Passive Latex Agglutination (RPLA) technique (SET-RPLA, Denka Seiken LTD, Japan) for the detection of these enterotoxins.

2.2.5. Antibiotic sensitivity of isolated *E. coli*, *Salmonella* and *Staph. aureus*

Antibiotic test was conducted on identified *Salmonella*, *E. coli* and *Staphylococcus aureus* on Mueller–Hinton agar (Oxoid) using disc diffusion method towards 12 antibiotics. The diameter of the zones of inhibitions was measured and classified as resistant and susceptible according to clinical and laboratory standard institute methods (CLSI, 2020).

3. RESULTS

Table 1. Incidence of *Salmonella*, *E. coli*, and *S. aureus* in examined chicken breast, thigh and liver samples.

Samples	No. of examined samples	Positive <i>Salmonella</i>		Positive <i>E.coli</i>		Positive <i>S. aureus</i>	
		No	%	No	%	No	%
Chicken breast	50	11	22	13	26	1	2
Chicken thigh	50	9	18	8	16	2	4
Liver	50	14	28	15	30	0	0
Total	150	35	23.3	36	24	3	2

Table 2. Serotypes of *Salmonella* species were identified from examined chicken meat and liver samples (35 isolates).

Type of sample	Identified serotypes	No. of Identified serotypes	%	Antigenic structure	
				O	H
Chicken Breast	<i>S. Typhimurium</i>	6	17	i = 1, 2	1, 4, 5, 12
	<i>S. Molade</i>	2	5.7	Z10:Z6	8,20
	<i>S. Enteritidis</i>	3	8.5	g, m: 1, 7	1, 9, 12
Chicken Thigh	<i>S. Typhimurium</i>	4	11.4	i = 1, 2	1, 4, 5, 12
	<i>S. infants</i>	2	5.7	r:1.5	6,7
	<i>S. kentucky</i>	3	8.5	i: Z6	8, 20
liver	<i>S. Typhimurium</i>	9	25.7	i = 1, 2	1, 4, 5, 12
	<i>S. kentucky</i>	2	5.7	i: Z6	8, 20
	<i>S. Entertidis</i>	4	11.4	g, m: 1, 7	1, 9, 12

Table 3. Serotypes of *Escherichia coli* were identified from examined chicken meat and liver samples (36 isolates).

Type of sample	Identified serotypes	No. of samples	%	Strain	Strain characterization
Chicken Breast	O127:H6	5	13.8	EPEC	Enteropathogenic
	O78	3	8.3	EPEC	Enteropathogenic
	O26:H11	1	2.7	EHEC	Enterohaemorrhagic
	O125: H21	4	11.11	ETEC	Enterotoxigenic
Chicken Thigh	O127:H6	3	8.3	EPEC	Enteropathogenic
	O119: H4	1	2.7	EPEC	Enteropathogenic
	O78	2	5.5	EPEC	Enteropathogenic
	O125: H21	2	5.5	ETEC	Enterotoxigenic
liver	O127:H6	4	11.11	EPEC	Enteropathogenic
	O119: H4	2	5.5	EPEC	Enteropathogenic
	O78	3	8.3	EPEC	Enteropathogenic
	O26:H11	2	5.5	EHEC	Enterohaemorrhagic
	O125: H21	4	11.11	ETEC	Enterotoxigenic

Table 4. Incidence of enterotoxins of *Staph. aureus* detected from examined chicken meat and liver samples (no =3).

Type of sample	No. of positive samples	%	Staph. aures toxins	Toxins
Breast	1	33.3	SEA	Staph enterotoxin type A
Thigh	2	66.6	SEA	Staph enterotoxin type A
			SEB	Staph enterotoxin type B
liver	0	0	-	-

Table 5. Sensitivity test for *salmonella* isolated from chicken meat and liver samples (no =35).

Antibiotic	Sensitive	%	Intermediate	%	Resistant	%
Colistin	30	85.7	0	0	5	14.2
Amoxicillin	0	0	0	0	35	100
Enrofloxacin	15	42.8	28.5	57	10	28.5
Doxycycline	5	14.2	5	14.2	25	71.4
Apramycin	0	0	0	0	35	100
Ofloxacin	0	0	4	11.4	31	88.5
Erythromycin	0	0	0	0	35	100
Cephadrine	0	0	0	0	35	100
Sulfamethoxazole	25	71.4	10	28.5	0	0
Ampicillin	0	0	5	14.2	30	85.7
Ciprofloxacin	5	14.2	6	17.1	24	68.5
Fosfomycin	18	51.4	10	28.5	7	20

Table (6): Sensitivity test for *E. coli* isolated from chicken meat and liver samples (no=36).

Antibiotic	Sensitive	%	Intermediate	%	Resistant	%
Colistin	25	69.4	5	13.8	6	16.6
Amoxicillin	0	0	0	0	36	100
Enrofloxacin	20	55.5	5	13.8	11	30.5
Doxycycline	14	38.8	12	33.3	10	27.7
Apramycin	6	16.6	0	0	30	83.3
Ofloxacin	4	11.11	2	5.5	30	83.3
Erythromycin	0	0	1	2.7	35	97.2
Cephadrine	0	0	2	5.5	34	94.4
Sulfamethoxazole	20	55.5	5	13.8	11	30.5
Fosfomycin	20	55.5	10	27.7	16	44.4
Streptomycin	0	0	0	0	36	100

Table (7): Sensitivity test for *Staph. aureus* isolated from chicken meat and liver samples (no=3).

Antibiotic	Sensitive	%	Intermediate	%	Resistant	%
Colistin	2	66.6	0	0	1	33.3
Amoxicillin	3	100	0	0	0	0
Ampicillin	0	0	1	33.3	2	66.6
Doxycycline	1	33.3	1	33.3	1	33.3
Enrofloxacin	1	33.3	1	33.3	1	33.3
Ofloxacin	0	0	2	66.6	1	33.3
Erythromycin	0	0			3	100
Cephadrine	0	0	1	33.3	2	66.6
Sulfamethoxazole	0	0	3	100	0	0
Fosfomycin	0	0	2	66.6	1	33.3

4. DISCUSSION

Contamination of poultry meat by enteropathogens remains a significant public health issue. Improper handling, inadequate cooking, and poor storage practices can lead to the transmission of pathogens, causing foodborne illnesses. In high-income countries, these illnesses result in increased morbidity, reduced workforce productivity, and substantial economic costs across food supply chains and healthcare systems. In low-income regions, the impact is even more severe, as foodborne infections exacerbate mortality rates, particularly among vulnerable populations with

preexisting health challenges (Moustafa et al., 2019).

The data in Table (1) indicates that the overall prevalence of *Salmonella*, *E. coli*, and *Staphylococcus aureus* in examined chicken breast, thigh, and liver samples was 35%, 24%, and 2%, respectively. The liver showed the highest incidence of *Salmonella* (28%) and *E. coli* (30%), likely due to internal contamination through blood, biliary, and lymphatic systems, or external contamination from improper handling (Barac et al., 2024). However, liver samples were free from *Staphylococcus aureus*. In breast and thigh samples, the prevalence of *Salmonella* was 22% and 18%,

respectively, while *E. coli* was detected at 26% and 16%. The prevalence of *Staphylococcus aureus* was lower, at 2% in breast samples and 4% in thigh samples.

Higher prevalence rates have been reported in some regions, such as Indonesia (85%) and Egypt, where the rates ranged from 33.3% to 50% in chicken carcasses and up to 60% in liver samples, likely due to internal and external contamination pathways (Yulistiani et al., 2019; Abdelrahman et al., 2020; Barac et al., 2024). Similarly, studies in Mansoura observed a 39% prevalence in chicken carcasses (Elshebrawy et al., 2022).

Lower prevalence of *Salmonella* in chicken meat was reported by Harb et al. (2018) who identified an 11.5% of *Salmonella* percent in chicken meat samples from Iraq. Similarly, Shaltout et al. (2020) reported *Salmonella* prevalence rates at 14% in chicken breast samples and 8% in chicken thigh samples collected from Ismailia city, Egypt.

Regarding to *E. coli* in chicken meat samples, these findings are consistent with previous studies, such as Elsenduony et al. (2022), which reported a 16.6% *E. coli* incidence in chicken thighs from Alexandria, and Brătfelan et al. (2023) which documented a 30% incidence in chicken meat from Romania.

A high prevalence of *Escherichia coli* in chicken meat has been documented in various studies, such as Khalafalla et al. (2015) who reported a 100% prevalence rate of *E. coli* in all examined thigh and breast samples. Similarly, Moustafa et al. (2019) observed contamination rates of 40% in chicken breast samples and 48% in thigh samples collected from the El-Boheria Governorate, Egypt. In contrast, ElBayoumi et al. (2018)

reported a lower prevalence of *E. coli* in 14.3% of chicken breast samples and 20% of thigh samples. Likewise, Shaltout et al. (2020) found contamination rates of 12% in chicken thigh samples and 18% in breast samples collected from Ismailia.

Regarding the prevalence of *Staphylococcus aureus* was 4% in chicken thigh samples and 2% in breast samples, resulting in an overall prevalence of 2% while in liver samples were completely free of *Staphylococcus aureus* (table 1).

Significantly higher prevalence rates of *Staphylococcus aureus* in chicken meat were recorded by Hamad et al. (2023) who documented rates of 84% in chicken thighs and 92% in breasts in Alexandria, Egypt. Similarly, Islam et al. (2024) observed 80% prevalence in chicken breast samples from Bangladesh. While in Mansoura, Egypt, Faraj et al. (2025) reported that 37.5% in chicken thighs and 12.5% in liver samples were recorded.

Table (2) highlights the diversity and prevalence of *Salmonella* serotypes isolates from 35 chicken meat and liver samples. *S. Typhimurium* was the most frequently detected serotype, with a prevalence of 25.7% in liver samples, 17% in chicken breast, and 11.4% in chicken thigh, indicating its dominant presence in chicken meat. Other notable serotypes included *S. Enteritidis*, found in 11.4% of liver samples and 8.5% of chicken breast samples, and *S. Kentucky*, detected in 8.5% of chicken thigh samples and 5.7% of liver samples.

Several studies confirm that *S. Typhimurium* is a dominant *Salmonella* serotype in chicken meat. Shaltout et al. (2019) reported that the incidence of 13.3% of chicken breast and 20% of

thigh samples were reported from Menofia Governorate, Egypt. In Ismailia City, Shaltout et al. (2020) identified *S. Typhimurium* (14.2%) and *S. Enteritidis* (71.4%) as the most common serotypes in chicken breast samples. Similarly, Elshebraway et al. (2022) found *S. Typhimurium* (24.3%), *S. Enteritidis* (19.1%), and *S. Kentucky* (16.4%) as the most prevalent serotypes in chicken carcasses. Barac et al. (2024) also revolved that *S. Typhimurium* was the predominant serotype in chicken meat.

Table (3) cleared that incidence of *E. coli* serotypes in chicken samples, as O₁₂₇:H₆ being the most prevalent, found in 13.8% of chicken breast, 8.3% of chicken thigh, and 11.11% of liver samples. Other notable serotypes include O₁₂₅:H₂₁ (11.11% in liver and 5.5% in both thigh and breast, classified as ETEC), O₇₈ (found in liver, thigh, and breast, classified as EPEC), and O₂₆:H₁₁ (2.7% in breast and 5.5% in liver, classified as EHEC). Additionally, O₁₁₉:H₄ was detected in 2.7% of thigh and 5.5% of liver samples, also classified as EPEC. These results are consistent with those of Shaltout et al. (2020), who reported the presence of *Escherichia coli* serotype O₁₂₇:H₆ in chicken samples. They observed an incidence rate of 16.6% in chicken breast samples and a higher rate of 33.3% in chicken thigh samples,

The O₁₂₇ strains of *Escherichia coli* have been identified as significant contributors to acute and persistent infantile diarrhea in developing countries (Levine, 1987). Additionally, the O₁₂₅:H₂₁ strains have been linked to human urinary tract infections, suggested that they may possess

uropathogenic characteristics (Abe et al., 2005).

Moreover, table (4) showed the distribution of *Staphylococcus aureus* enterotoxins in chicken meat samples. The findings indicate that enterotoxin A (SEA) was produced exclusively by *S. aureus* isolates from chicken breast samples. In contrast, two isolates from chicken thigh samples produced both enterotoxins A (SEA) and B (SEB), highlighting the presence of multiple enterotoxins in certain samples. The findings indicate that *Staphylococcus aureus* enterotoxin A (SEA) is the most produced enterotoxin, followed by enterotoxin B (SEB). This aligns with the observations of Darwish et al. (2018), who also identified SEA as the predominant enterotoxin produced by *S. aureus* isolated from chicken meat, with SEB being the second most common.

Staphylococcus aureus food poisoning is a foodborne illness caused by the ingestion of foods contaminated with performed enterotoxins. It is characterized by a rapid onset of symptoms, typically occurring within 3 to 6 hours after consumption. Common symptoms include vomiting, which may be accompanied by diarrhea, and abdominal pain. In severe cases, these symptoms can lead to dehydration and even shock (Hennekinne et al., 2012).

Furthermore table (5) revealed complete resistance (100%) of *salmonella* to Amoxicillin, Apramycin, Erythromycin, and Cephadrine. High resistance levels were also observed for Ofloxacin (88.5%) and Ampicillin (85.7%). Conversely, the isolates showed notable sensitivity to Colistin (85.7%), Sulfamethoxazole (71.4%),

Enrofloxacin (42.8%), and lower sensitivity to Ciprofloxacin and Doxycycline (14.2% of each). The universal resistance to Erythromycin is particularly concerning, given its frequent use in treating avian diseases, especially in Egypt (Saadeldin & Reda, 2016).

The findings of present study align with previous research on antimicrobial resistance in *Salmonella* isolates as Adeyanju and Ishola (2014) who reported 100% resistance to amoxicillin, while Abd-Elghany et al. (2015) observed 100% resistance to both erythromycin and amoxicillin, with ampicillin resistance reaching 91.6%. Similarly, Moawad et al. (2017) found that 86.7% of *Salmonella* isolates were resistant to ampicillin.

The antimicrobial sensitivity analysis of *E. coli* isolates in table (6) revealed alarming resistance patterns. All isolates (100%) were resistant to amoxicillin and streptomycin, with high resistance rates observed for erythromycin (97.2%), cephradine (94.4%), and both apramycin and ofloxacin (83.3%). However, some antibiotics showed promising sensitivity, with 69.4% of isolates being sensitive to colistin and 55.5% showing sensitivity to enrofloxacin, sulfamethoxazole, and fosfomycin.

The findings of the present study align with previous research on antimicrobial resistance in *Escherichia coli* isolates, as Adeyanju and Ishola (2014) who reported that 100% resistance rate to amoxicillin in *E. coli* isolates from retail chicken meat. Similarly, Ahmed et al. (2009) observed high resistance rates to multiple antibiotics, including

ampicillin, streptomycin, spectinomycin, kanamycin, tetracycline, and trimethoprim/sulfamethoxazole.

Moawad et al. (2017) also observed high resistance to amoxicillin and streptomycin in *E. coli* isolates from chicken meat in northern Egypt, with all isolates being sensitive to colistin. In contrast, Mohamed et al. (2014) reported 100% resistance to gentamicin.

The antimicrobial sensitivity analysis of three *Staphylococcus aureus* isolates revealed a distinct resistance and sensitivity patterns. All isolates (100%) were resistant to erythromycin, while 66.6% exhibited resistance to both ampicillin and cephradine. Conversely, all isolates (100%) were sensitive to amoxicillin, with 66.6% showing sensitivity to colistin and 33.3% to doxycycline and enrofloxacin.

The findings of the study also are consistent with previous research on antimicrobial resistance in *Staphylococcus aureus* isolates, as Darwish et al. (2018) who reported that 78.57% of *S. aureus* isolates from Zagazig City, Egypt, exhibited resistance to ampicillin, while 21.4% were resistant to erythromycin. Similarly, Islam et al. (2024) observed that 88.46% of *S. aureus* isolates in Bangladesh demonstrated resistance to ampicillin.

In conclusion, the study underscores critical food safety challenges in Menoufia poultry supply chain, with *Salmonella*, *E. coli*, and *Staphylococcus aureus* contamination, especially in chicken liver. The presence of virulent serotypes such as *Salmonella* Typhimurium and enterotoxigenic *E. coli* O127:H6,

combined with alarming antibiotic resistance patterns, poses significant risks of zoonotic transmission and treatment failures. Liver samples, identified as key reservoirs for pathogens, require prioritized scrutiny. The widespread resistance to first-line antibiotics like amoxicillin and erythromycin reflects systemic misuse in poultry farming, exacerbating the threat of antimicrobial resistance (AMR). Effective interventions are necessary to mitigate these risks, including enhanced food safety protocols throughout the supply chain and stricter regulations on antibiotic use in poultry production.

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