Daagu International Journal of Basic and Applied Research 7(1) (2025): 616-625

Research Article

Prevalence and Antimicrobial Resistance Profile of Salmonella Isolated from Chicken Eggs Purchased from Open Markets in Afar Region, Ethiopia

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Received: November 3, 2024 Revised: April 05, 2025 Accepted: May 26, 2025

Abstract

A cross-sectional study was conducted from January to May 2020 with the objectives of estimating the prevalence of Salmonella isolates from chicken eggs purchased at the open markets of Asayita and Dubti towns in the Afar region of Ethiopia and evaluating their antimicrobial susceptibility patterns. A total of 384 chicken egg samples were purchased using a simple random sampling method from the local open markets of Dubti and Asayita towns. A swab technique was used to sample the shell surface of the intact eggs. The egg contents were homogenized in stomacher bags containing 225 mL of buffered peptone water. Descriptive statistics were employed to analyze the data. Out of the 384 eggs purchased, 68 (17.70%) samples from the eggshell were found to be positive for Salmonella, and 26 (6.77%) samples were found to be positive from the internal egg contents. The overall prevalence of Salmonella in eggshells was significantly higher than that of Salmonella from the egg contents. Of the 26 Salmonella isolates subjected to antimicrobial susceptibility tests, 12 (46.2%) were found to be resistant to one or more antimicrobials, 8 (30.8%) isolates showed intermediate sensitivity to one or more antimicrobials, and 6 (23.1%) isolates were susceptible to all tested antimicrobials. Among the isolates tested, 34.6%, 23.1%, and 11.5% of the Salmonella isolates were found to be relatively resistant to tetracycline, sulfamethoxazole-trimethoprim, and ampicillin, respectively. The present study findings revealed the presence of Salmonella species both from eggshells and egg contents. Further studies should be conducted to identify the Salmonella species involved, which is crucial for recommending appropriate antibiotic therapy in suspected Salmonella cases.

Key words: Afar, Antimicrobial Susceptibility, Chicken Egg, Prevalence, Salmonella isolates

1. INTRODUCTION

Foodborne pathogens are among the most widespread global public health problems, and their impact on national economies is increasingly recognized. Many individuals suffer from foodborne diseases worldwide

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due to contaminated food and water consumption (Hendriksen et al., 2007; Majowicz et al., 2010). Among the pathogens, salmonella is considered the most prevalent foodborne pathogen globally and has been identified as a major foodborne disease that poses a serious public health problem, particularly in developing countries (Sánchez-Vargas *et al*., 2011; Carrasco *et al*., 2012). The National Hygiene and Sanitation Strategy program (MoH, 2005) reported that in Ethiopia, more than 250,000 children die every year from sanitation and hygiene-related diseases, and about 60% of the disease burden is linked to poor hygiene and sanitation practices. The primary source of infection is food of animal origin, such as poultry meat, eggs, milk, beef, and pork. Poultry meat, eggs, and egg products are among the most nutritious foods on earth and play an important role in the human diet. However, like other perishable items, improperly handled poultry and egg products are closely associated with foodborne illnesses, such as salmonellosis (Shahzad, 2012). Salmonellosis is an infectious and economically significant disease affecting humans and animals caused by Salmonella species (OIÉ, 2000).

Eggs and egg products had been incriminated in foodborne outbreaks (EFSA, 2010). Despite the nutritional value of eggs, there are some potential health issues arising from cholesterol content, *Salmonella* contamination and allergy to egg proteins. *Salmonella* is capable to cause a variety of disease syndromes: such as enteric fever, bacteremia, enterocolitis, and focal infections. This pathogen that causes enteric fever and gastroenteritis in humans and animals (Didelot *et al.,* 2011).

The presence of Salmonella species in poultry and poultry products, including eggs, is a global public health concern including Ethiopia. Salmonella causes gastrointestinal infections that are accompanied by inflammation of intestinal epithelia, diarrhea and vomiting, to typhoid fever. Various contamination levels have been reported by numerous studies worldwide: A study finding in Addis Ababa revealed that a prevalence of 5.21% Salmonella enteritidis from eggs purchased from local markets (Bayu *et al*., 2013). A prevalence of 5.3% has been found from eggs shells from the open market purchased eggs in and around Haramaya Town (Kemal *et al*., 2016) and a prevalence of 18% from eggs in Gondar. Moreover, a study conducted by Wiriya *et al.* (2011) reported a prevalence of 5.2% from egg shells. One study in Ethiopia found that from a total of 400 chicken eggs examined for Salmonella, 46 (11.5%) were positive; 6.3% and 6.8% of isolates were found on egg shells and in egg contents, respectively (Minte *et al*., 2011). A prevalence of 8% from chicken eggs in Dhaka city (HKSAR, 2004) and 24% in Nigeria (Gast, 2006). Antibiotic-resistant *Salmonella* infections of both human and animals are universal concerns. Antimicrobial-resistant *Salmonella* spp. have been isolated from different foods of animal origin around the world, which is attributed to the inappropriate use of antimicrobials as therapeutic or prophylactic agents in human and veterinary medicine, as well as the use of growth promoters in animal production (WHO, 2012). There is very limited data available on the *Salmonella* isolated from chicken eggs in the country though the disease

is nationally as well globally a concern and a leading zoonotic pathogen. Here in Afar region, no single data was reported about the presence of *Salmonella* from eggs purchased from local markets in selected towns of the region. The present study, therefore, was aimed to determine the prevalence of Salmonella from chicken eggs purchased from retailers in the outlets of selected markets and evaluated antimicrobial resistance pattern of the isolates in selected cities of Afar region, Ethiopia.

2. MATERIALS AND METHODS

2.1. Description of the Study Area

Afar National Regional State is one of the eleven federal states of Ethiopia, located in the north-eastern part of the country and it is 588 kilometers (km) from the capital city of Ethiopia (Figure 1). It is one of the four major pastoral regions in Ethiopia. The annual temperature and rainfall in the region is 30-50^{0}c and 200-600mm, respectively. The altitude in the region is from 100-1000 meters above sea level. People in the region mainly depend on livestock production for their livelihood. The study sites are Asayita and Dubti districts. The average elevation in this woreda is 503m above sea level (m.a.s.l) (Hailu, 2008).

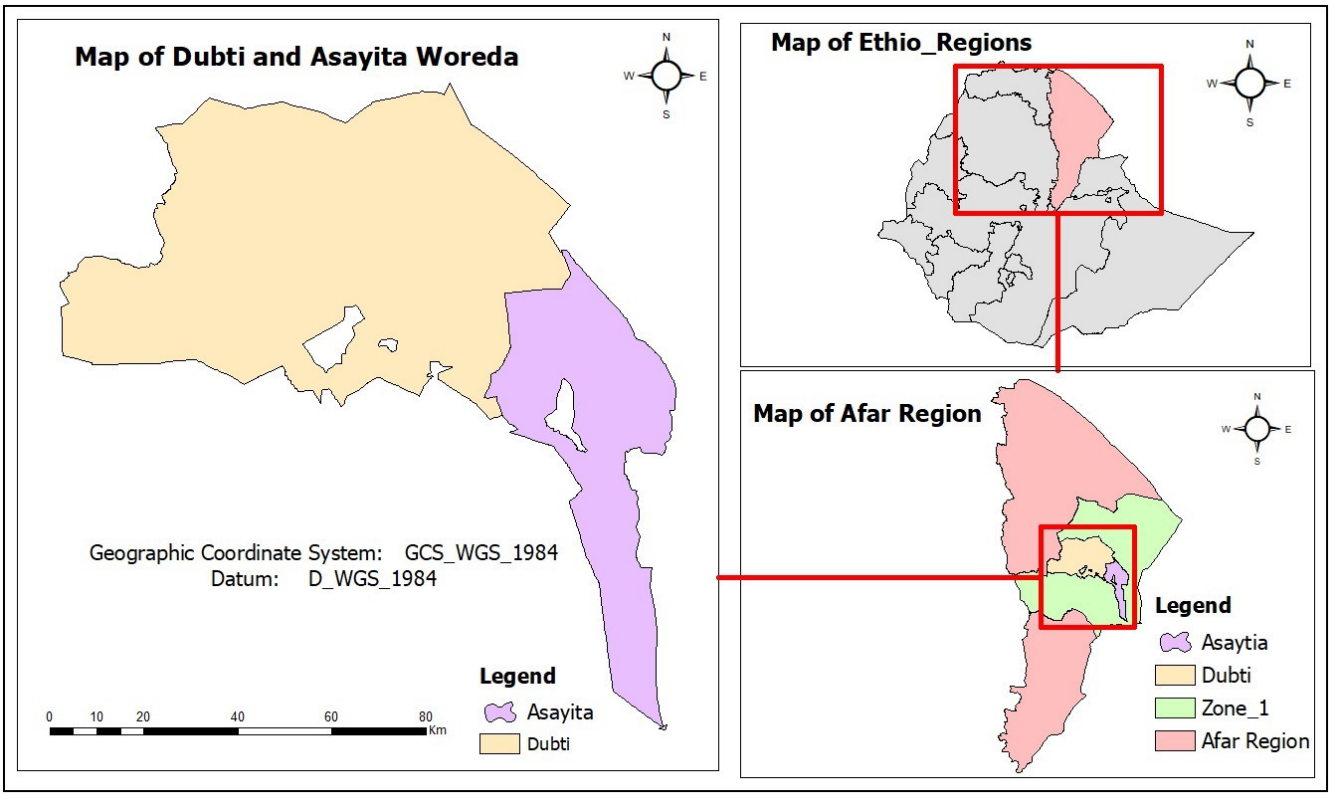


Figure 1: Map of Afar Region indicating the study districts (Source: Adapted from Ethiopia mapping agency)

2.2.Study Design and Population

A cross-sectional study was conducted during the study period from January to May, 2020 to estimate the prevalence of Salmonella isolates from chicken eggs (shell and contents) and determine the antimicrobial resistance pattern of the isolates.

2.2.1. Sampling Technique and Sample Size Determination

The sample size was determined using the expected prevalence of 50% Salmonella isolates, a 95% confidence level, and a 5% desired precision level, according to Thrusfield (2007). Consequently, a total of 384 chicken egg samples were collected from local sellers at the retail outlets in the open markets of Dubti and Asayita districts. The egg samples were collected using a simple random sampling technique. After purchase, the egg samples were labeled and placed into an egg container bag for transport to the Kombolcha Livestock Disease Survey, Investigation, and Diagnostic Regional Laboratory for the isolation and identification process.

2.2.2. Isolation and Identification of Salmonella Isolates

The isolation and identification of the bacteria was conducted according to the method described by the International Organization for Standardization (ISO 6579:2002). A swab technique was used to sample the shell surface of the intact eggs. Sterile cotton swabs dipped into sterile buffered peptone water (BPW) were used to swab the entire surface area of the egg shell. The swabs were then incubated separately in a test tube that contained 10mL BPW (Oxoid Ltd, Hampshire, UK; Lab M Ltd., Quest Park, UK). The egg contents were homogenized in stomacher bags containing 225mL sterile BPW for 1minute in a stomacher. The isolation was conducted using the conventional methods for the detection of *Salmonella*, following the standard guidelines from ISO 6579. After pre-enrichment 0.1 ml of the broth was transferred in to a test tube containing 10ml of Rappaport-Vassiliadis (RV) broth (HiMedia Laboratories Pvt. Ltd, Mumbai, India) and another 1ml was transferred into a tube containing 10ml of tetrathionate broth (Titan Biotech Ltd, Rajasthan, India). The inoculated RV broth was incubated at 42°C for 24hrs and the inoculated tetrathionate broth at 37 °C for 24 hrs. Colonies incubated for 24hrs until it became turbid were then transferred to XLD agar using streaking loop and was incubated for 24hrs at 37°C. *Salmonella* suspected colonies from XLD agar medium were plated onto nutrient agar (Oxoid, England) plates to use for biochemical confirmation. Each identified colony with typical *Salmonella* morphology was confirmed biochemically by inoculating into triple sugar iron (TSI) agar, methyl red-Voges-Proskauer (MR-VP) broth, indole test, Simmons' citrate agar, urea agar, and lysine iron agar (LIA) incubated at 37ºC for 24 hours and interpreted with the International Organization for Standardization guidelines (ISO 6579:2002).

2.2.3. Antimicrobial susceptibility test

Salmonella isolates were subjected to in-vitro susceptibility test against commonly used antimicrobial agents using disk diffusion method following guidelines established by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2012). In brief, by taking pure isolated colony, bacterial suspension was adjusted to 0.5McFarland turbidity standards. The diluted bacterial suspension was then transferred to Mueller-Hinton agar plate using a sterile cotton swab and the plate was seeded uniformly by rubbing the swab against the entire agar surface followed by 24 h incubation. After the inoculums were dried, antibiotic impregnated disks were applied to the surface of the inoculated plates using sterile forceps. The plates were then incubated. Finally, the zone of inhibition was measured including the disk diameter. The susceptible, intermediate and resistant categories were assigned on the basis of the critical points recommended by the CLSI and according to the manufacturer's leaflet attached to the disks. Antimicrobials used for susceptibility testing of Salmonella species were Amoxicillin (AML-2µg), Ampicillin (AMP-10 µg), Tetracycline (TE-30µg), Gentamicin (CN-10µg), Sulfamethoxazole-Trimethoprim (SXT-25µg), Ceftriaxone (CRO-30µg), Nitrofuranthoin (F-100µg), and Nalidixic acid (NA-30µg).

2.3.Data analysis

The data were filled using Microsoft office Excel 2007, then descriptive statistics and prevalence were used to analyze the data manually. The prevalence was calculated by dividing the number of positive samples on biochemical test by the total number of eggs sampled.

3. RESULTS

3.1. Prevalence of Salmonella isolates from chicken egg shell and egg contents

In the current study, out of the 384 egg samples purchased from Asayita and Dubti town open local markets for bacteriological identification, 68(17.70%) samples from egg shell (43 (11.19%) samples from Asayita, and 25 (6.51%) samples from Dubti towns were found to be positive for *Salmonella* in egg shells. Twenty-six (6.77%) samples were found to be positive for Salmonella from internal egg contents (15 (3.91%) egg samples from Asayita and 11 (6.77%) eggs samples from Dubti towns). The overall prevalence of *Salmonella* in egg shells was higher than that of *Salmonella* from egg contents. The numbers of positive samples before and after biochemical tests are shown in (Table 1).

Table 1: Isolation and Biochemical characterization of salmonella isolates from chicken eggs.

Sample code	XLD	BGA	Indole	TSI	Citrate	Urease	MR	VP	Result
A-EC-S8	Pink w BC	Pink	-	+	+	-	+	-	*Salmonella spp.*
A-EC-S16	Pink w BC	Red	-	+	+	-	+	-	*Salmonella spp.*
A-EC-S29	Pink w BC	Red	-	+	+	-	+	-	*Salmonella spp.*
A-EC-S42	Pink w BC	Pink	-	+	+	-	+	-	*Salmonella spp.*
A-EC-S48	Pink w BC	Red	-	+	+	-	+	-	*Salmonella spp.*
A-EC-S54	Pink w BC	Pink	-	+	+	-	+	-	*Salmonella spp.*
A-EC-S82	Pink w BC	Red	-	+	+	-	+	-	*Salmonella spp.*
A-EC-S87	Pink w BC	Red	-	+	+	-	+	-	*Salmonella spp.*
A-EC-S101	Pink w BC	Pink	-	+	+	-	+	-	*Salmonella spp.*
A-EC-S121	Pink w BC	Pink	-	+	+	-	+	-	*Salmonella spp.*
A-EC-S146	Pink w BC	Pink	-	+	+	-	+	-	*Salmonella spp.*
A-EC-S161	Pink w BC	Red	-	+	+	-	+	-	*Salmonella spp.*
A-EC-S163	Pink w BC	Red	-	+	+	-	+	-	*Salmonella spp.*
A-EC-S184	Pink w BC	Red	-	+	+	-	+	-	*Salmonella spp.*
A-EC-S207	Pink w BC	Pink	-	+	+	-	+	-	*Salmonella spp.*
D-EC-S218	Pink w BC	Red	-	+	+	-	+	-	*Salmonella spp.*
D-EC-S237	Pink w BC	Red	-	+	+	-	+	-	*Salmonella spp.*
D-EC-S265	Pink w BC	Red	-	+	+	-	+	-	*Salmonella spp.*
D-EC-S268	Pink w BC	Pink	-	+	+	-	+	-	*Salmonella spp.*
D-EC-S289	Pink w BC	Pink	-	+	+		+	-	*Salmonella spp.*
D-EC-S314	Pink w BC	Red	-	+	+	-	+	-	*Salmonella spp.*
D-EC-S315	Pink w BC	Pink	-	+	+	-	+	-	*Salmonella spp.*
D-EC-S353	Pink w BC	Pink	-	+	+	-	+	-	*Salmonella spp.*
D-EC-S368	Pink w BC	Red	-	+	+	-	+	-	*Salmonella spp.*
D-EC-S369	Pink w BC	Red	-	+	+	-	+	-	*Salmonella spp.*
D-EC-S372	Pink w BC	Red	-	+	+	-	+	-	*Salmonella spp.*

Key: BC = Black Center; XLD = xylose lysine deoxycholate; BGA= Brilliant green agar; TSI = Triple Sugar agar; MR= Methyl red; VP = Voges-Proskauer

The prevalence of Salmonella across the study areas is presented in (Table 2) below.

Table 2: Prevalence of *Salmonella* from chicken eggs content purchased from local market.

Sample Collection Site	No of samples examined	Egg shell	Egg content
		No of positive	No of positive
Asayita	225	43(11.19%)	15(3.91%)
Dubti	159	25 (6.51%)	11(2.86%)
Total	384	68(17.7%)	26 (6.77%)

3.2. Antimicrobial Susceptibility Patterns of Salmonella Isolates

Salmonella isolates were subjected to in-vitro susceptibility test against commonly used antimicrobial agents using disk diffusion method following guidelines established by the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2012). The overall proportion of antimicrobial susceptibility test and the results of the antimicrobial resistance profile of *Salmonella spp.* isolated from chicken eggs are given in

(Table 3). Of the total 26 *Salmonella* isolated from egg contents subjected to antimicrobial susceptibility test using 10 different antimicrobials, 12(46.2%) *Salmonella* isolates were found to be resistant to one or more than one of the antimicrobials, and 8(30.8%) isolates showed an intermediate sensitivity to one or more antimicrobials, and 6 (23.1%) isolates were susceptible to all tested antimicrobials.

Table 3: The general proportion of antimicrobial susceptibility test among *Salmonella* isolates.

Antimicrobials	**Antimicrobial susceptibility test**			**Remarks**
	Sensitivity (%)	Intermediate (%)	Resistant (%)	
Amoxicillin (AML)	17(65.4)	4(15.4)	3(11.5)	
Ampicillin (AMP)	18 (69.2)	3(11.5)	2(7.7)	
Cephalothin (KF)	15 (57.7)	3(11.5)	2(7.7	
Ceftriaxone (CRO)	21 (80.8)	0	0	
Gentamicin (CN)	22 (84.6)	0	2(7.7)	
Nalidixic acid (NA)	19(70.1)	0	3(11.5)	
Nitrofurantoin (F)	5(19.2)	4 (15.4)	2(7.7)	
Sulfamethoxazole-trimethoprim (SXT)	8 (30.8)	0	6(23.1)	
Tetracycline (TE)	10(38.5)	2 (7.7)	9(34.6)	

Concerning the total *Salmonella* isolates tested, 34.6%, 23.1%, and 11.5% were found to be relatively resistant to tetracycline, sulfamethoxazole-trimethoprim, and ampicillin and Nalidixic acid, respectively, while 7.7%-15.4% were intermediately resistant to tetracycline, amoxicillin, ampicillin, cephalothin, and nitrofurantoin. Looking at individual antimicrobials, resistance to tetracycline and sulfamethoxazole-trimethoprim was frequently observed, followed by ampicillin, amoxicillin, gentamicin, nalidixic acid, and nitrofurantoin. In general, antimicrobial susceptibility tests revealed that ceftriaxone, gentamicin, and nalidixic acid were the antimicrobials indicated as active against *Salmonella spp*. isolated from this study.

4. DISCUSSION

Salmonella is one of the most significant genera of pathogenic bacteria implicated in foodborne illnesses. Egg-associated salmonellosis is a critical public health issue in Ethiopia and other countries when eggs are consumed raw or undercooked (FAO, 2017). In the present study, the prevalence and antimicrobial resistance patterns of Salmonella spp. isolated from chicken eggs purchased at open local markets in Asayita and Dubti towns were assessed. Of the 384 egg samples purchased from the open local market and examined for bacteriological status, 68 (17.70%) samples from the eggshell were found positive for Salmonella, and 26 (6.77%) chicken egg samples were positive for Salmonella from the internal egg contents. Regarding the prevalence of Salmonella from the eggshell, this research's result is significantly higher than the previous study findings of 6.3% by Minte *et al*. (2011), 5.3% by Kemal *et al*. (2016), and 5.2% by Wiriya *et al.* (2011), which reported varying Salmonella prevalence rates from the eggshell. However, the current study's finding regarding the prevalence of Salmonella from chicken egg contents aligns closely with previous

research findings by Minte *et al*. (2011), which reported a prevalence of Salmonella in egg contents of 6.8%, nearly equivalent to our finding of 6.77%. Moreover, an overall prevalence of Salmonella has been reported as 8% from chicken eggs in Dhaka City (HKSAR, 2004) and 24% in Nigeria (Gast, 2006). The prevalence of Salmonella in this study may be attributed to the level of environmental contamination during storage, the health status of the chickens, transportation, and ineffective cleaning of eggs.

A study conducted by Kohinur *et al*. (2010) reported that 100% of strains isolated from poultry eggs were sensitive to ciprofloxacin and chloramphenicol. In contrast, various Salmonella isolates demonstrated resistance to ampicillin, tetracycline, and kanamycin. Chen *et al*. (2004) analyzed 133 Salmonella isolates and found that 73 (82%) of these isolates were resistant to at least one antimicrobial agent. Resistance was particularly common to tetracycline (68%) and ampicillin (29%). This issue is likely linked to the widespread use of these antimicrobials in both humans and animals for treating enteric infections. The current study's findings support previous research indicating that Salmonella isolates from chicken eggs exhibited higher resistance to tetracycline (34%), followed by sulfamethoxazole-trimethoprim (SXT) (23.1%). Sulfamides, fluoroquinolones, and tetracyclines are the most prevalent antimicrobials used in chicken flocks across many countries, including Spain (Capita *et al*., 2007). Consequently, a high level of resistance to tetracyclines, as well as to sulfamethoxazole, was observed. Resistance to nalidixic acid and amoxicillin was found at a rate of 11.7%. Other studies on Salmonella isolates from poultry (Capita *et al*., 2007; Voss-Rech *et al*., 2015) noted similar resistance patterns to these antimicrobial groups. These data suggest that using these antimicrobials in broilers can contribute to the emergence of resistant strains. In this study, one of the most common resistances was to tetracycline (34.1%), mirroring previous findings by Usera *et al*. (2002) and Carramiñana *et al*. (2004), although significant resistance to sulfamethoxazole-trimethoprim (SXT) and chloramphenicol was also observed.

5. CONCLUSION AND RECOMMENDATIONS

The present study indicated that *Salmonella* isolates were identified from the shell and internal contents of raw chicken eggs sold on open local markets. *Salmonella* antimicrobial resistance was also observed for almost all tested antibiotics that are commonly used in both veterinary and human health sectors in the study area and other parts of Ethiopia. Therefore, our findings provided the prevalence and drug resistance of *Salmonella* from chicken egg and contribute information to scientists as well as public health researchers for the development of guidelines as well as food safety regulations to minimize the prevalent and resistant foodborne *Salmonella* species. Further studies should be carried out to identify which salmonella species involved to the occurrence of the disease.

FUNDING

This research work was financially supported by Samara University, Research and technology transfer vice president office.

ACKNOWLEDGMENTS

The authors would like to thank research and technology transfer office of Samara University and Kobmolcha regional veterinary laboratory for the provision of laboratory facilities and space to execute this research work.

Authors' contributions

TD: Contributed to conception of the research idea, designing and data collection, data analysis, interpretation of data, writing and editing of the manuscript. WN: Contributed to the study concept, sample collection, interpretation of data. Both authors read and approved the final manuscript for publication.

Availability of data and materials

The data sets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Consent for publication

Not applicable

Competing interests

The authors declare that they have no competing interests in the publication of this paper.

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