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Research Article

Microbial Profile and Safety of Chicken Eggs from a Poultry Farm and Small-Scale Vendors in Hawassa, Southern Ethiopia

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A freshly laid hen's egg is devoid of microorganism, but soon after oviposition, it is contaminated by various spoilage and pathogenic microorganisms. The aim of this study was to assess the microbial profile and safety of chicken eggs in Hawassa City. A total of 60 egg samples were collected from Hawassa University Poultry Farm (HUPF) and small-scale vendors in Hawassa. The samples were analyzed for aerobic mesophilic bacterial count (AMBC), *Staphylococcal* count (SC), *Enterobacteriaceae* count (EC), total coliform count (TCC), fecal coliform count (FCC), and yeast and mold count (YMC). Moreover, the dominant mesophilic aerobic bacterial genera and common bacterial pathogens were identified by phenotypic methods. Accordingly, the mean aerobic mesophilic bacterial load of the shell surface rinsate of the egg samples ranged from 1.22 $\log_{10}$ CFU/ml to 9.7 $\log_{10}$ CFU/ml, while that of the internal contents ranged from 1.52 log CFU/ml to 9.36 log CFU/ml. The microbial load values of the egg contents were beyond the international recommended acceptable limits and suggested incipient spoilage. The mesophilic aerobic bacterial genera of the shell and internal contents of the egg samples were similarly dominated by *Pseudomonas*, *Micrococcus,* and *Staphylococcus*. The incidence of *E. coli* in shell rinsate and egg content was 10% (6 of 60) and 1.67% (1 of 60), respectively. Salmonellae were detected in shell rinsate of six egg samples (10%) and in the contents of eight samples (13.33%), all from small-scale vendors. These findings call for vigilant exercise of good agricultural and hygienic practices by primary producers and retailers.

1. Introduction

Chicken egg is one of the most nutritious and versatile human foods. On average, it consists of 10% shell, 58% albumin (white), and 32% yolk [1]. Nutritionally, on average, whole freshly laid egg consists of 76.1% water, 12.6% protein, 9.5% fat, 0.7% carbohydrates, and 1.1% ash [2, 3]. Egg protein is one of the highest quality proteins with more than 90% bioavailability [4]. Moreover, compared with other sources of animal protein, eggs are the most affordable ones, making them a sustainable means of supporting optimal development and reducing malnutrition in children. Eggs are versatile ingredients in different types of dishes and are used for coagulation, foaming, emulsifying, coloring, and flavoring. While there are some claims implicating egg diet as a factor for cardiovascular diseases [5], the nutritional and other health benefits far outweigh the risks [6]. Therefore, the egg remains a food product of high nutritional quality and is consumed worldwide.

Despite all the nutritional and economic attractions of the egg sector, there are also associated challenges, especially the risk of transmission of food-borne microbial diseases and spoilage [7]. Contaminated eggs have been incriminated as the major cause of foodborne salmonellosis [8]. Contaminated eggs accounted for 53% of all cases of Salmonella in the United States between 1985 and 2002 [9, 10]. A freshly laid hen's egg is generally devoid of microorganism, but soon after oviposition, the shell surface becomes contaminated by

various spoilage and pathogenic microorganisms [10, 11]. The sources of eggshell microbial contamination may include the fecal matter, the nesting material, the feed, air and the collecting person, or the storage equipment [12]. Moreover, eggs can also be inherently colonized from the natural flora of the laying hen.

The eggshell has the highest bacterial contamination as it is the outermost exposed part. On average, the microbial load of the eggshell with regard to aerobic mesophilic bacterial count may range between 3.8 and 6.3 $\log_{10}$ CFU/egg [13]. The most commonly encountered microbial contaminants include *Pseudomonas, Alcaligenes, Proteus mirabilis, Salmonella* typhimurium, *Salmonella* Dublin, *Salmonella* braenderup, *Citrobacter, Escherichia coli, Enterobacter cloacae, Klebsiella, pneumonia*, *Enterococcus faecalis, Micrococcus*, *Staphylococcus* species, *Bacillus,* and *Stenotrophomonas maltophilia* [14]. By virtue of their resistance to harsh conditions, molds are also important spoilage organisms of egg stored for long period [14, 15].

Contaminating microorganisms that enter the egg content must hurdle different barriers [15, 16]. The first step is adherence to the eggshell and then passage through the eggshell pores into the interior. Inside the egg, the components of the egg white (lysozyme, conalbumin, avidin, and alkaline pH of the albumen) present harsh environment that prevents the proliferation of microbes [14, 15]. Therefore, invading microbes must overcome the antimicrobial components of the albumen and survive to reach the egg yolk where they metabolize and multiply to cause spoilage [7]. The egg yolk presents no challenge but provides an excellent growth medium for contaminating microorganisms.

The most common contaminants that penetrate the egg content include *Salmonella* typhimurium, *E. coli,* and *P. aeruginosa,* which have flagella that allow them to penetrate through the pores [7]. Salmonellae are also known to be deposited directly into the developing yolk from an infected ovary of a laying hen [14, 16]. *S. enterica* serotypes typhimurium and enteritidis are the two most commonly identified causative agents of foodborne salmonellosis [17]. Both serotypes have the ability to colonize the reproductive organs of hens (oviduct and ovary) and are major causes of foodborne illness.

In Ethiopia, poultry and egg production play important role in creating job opportunities, generating lucrative income, and tackling malnutrition [18]. Despite this, Ethiopia has one of the lowest levels of egg availability in all of Africa, at just 8 eggs and 13 eggs per person per annum in rural and urban areas, respectively [19], very low as compared to a global average of 180. Low egg production in Ethiopia is attributed to the predominance of the backyard rearing of indigenous breeds characterized by low productivity, high mortality rates, poor nutrition, and supply chain.

In addition to the low productivity, a significant amount of the production is lost to microbial spoilage due to unhygienic handling in the egg chain. There is no working legislation or guideline to protect the consumers in the market from poor quality and hazardous or contaminated products. Egg grading or labeling is rarely done, and it is up to the consumer to check the soundness of the product during the transaction in the market. Besides, there exists in many communities in Ethiopia a culture of drinking raw egg as a medication for respiratory and other illnesses. Although surveillance data are lacking on egg-borne salmonellosis, the prevalence of up to 11% on table eggs has been reported [4]. Therefore, this study aimed to investigate the microbial profile and safety of raw shell eggs marketed in Hawassa City, southern Ethiopia.

2. Materials and Methods

The study was done in Hawassa City, the administrative center of Sidama and Southern Nations, Nationalities, and People's Region (SNPPR) in Ethiopia.

2.1. Study Design. A cross-sectional study design was employed based on the laboratory analysis of chicken egg samples collected from open market and retail outlet of a poultry farm during the period between February 2019 and November 2019.

2.2. Sample Size and Sampling Techniques. Arbitrarily, a total of 60 eggs were considered in the study consisting of 15 egg samples from the HU Poultry Farm (HUPF) and nine samples from each of five randomly selected retail shops located in five different city zones in Hawassa (Table 1).

Eggs marketed in the retail outlets in Ethiopia in general are not graded or labeled with regard to size, production date, or age. It is up to the consumer to check the soundness of the produce at the time of transaction. On the other hand, eggs from the HUPF were not older than two days. Therefore, from each of the sampling points three eggs were randomly purchased and transported at ambient temperature (ca. 25°C) in sterile plastic bags to the Microbiology Laboratory of the Department of Veterinary Medicine, HU, and microbiological analysis was done on the same day of the sample collection.

2.3. Preparation of Sample from the Eggshell. This was done according to the surface rinse method [20, 21]. Briefly, each egg sample was immersed and washed in sterile 100 ml of normal saline (0.85%) aqua solution in a 300 ml capacity beaker by shaking gently and then allowed to stand for 10 minutes. After that, the egg was removed and the resulting rinsate was considered as 10^{-2} dilution of the shell surface flora. From this, further tenfold dilution was prepared up to 10^{-7} by transfer of 1 ml aliquots into tubes of dilution blanks containing 9 ml sterile normal saline solution as diluent. Tubes were vortex mixed between transfers to ensure uniform homogeneity as described before.

2.4. Preparation of Sample from the Internal Egg Content. Microbial contamination of the internal contents of eggs inherently from an infected hen *via* transovarian and oviducal way or trans-shell migration by motile microbes from external sources is a well-known phenomenon. Therefore, each rinsed egg sample as treated above was further

Table 1: Sampling locations and sample size of shell eggs considered in the study of microbial profile and safety in Hawassa City, southern Ethiopia.

Sampling location	Number of egg samples
HU Poultry Farm	15
Wukro Kebele	9
Atote	9
Menahariya	9
Addis Ketema	9
Bahil Adarash	9
Total	60

immersed in sufficient amount of absolute ethanol in a beaker and drained off, and the surface of the shell is flamed by passing through the flame of the Bunsen burner. After that, it was carefully cracked with the sterile spatula and the whole egg content (white along with the yolk) was aseptically transferred into another sterile beaker and mixed thoroughly by stirring with sterile spatula until uniform homogeneity was attained. From this homogenate, 10 ml sample was aseptically transferred using the sterile pipette into a bottle containing 90 ml of normal saline solution diluent and mixed thoroughly by vortexing for about 10 minutes [10]. From this 10^{-1} dilution, further tenfold serial dilutions were prepared up to 10^{-6} [20, 21].

2.5. Media and Sample Preparations and Microbial Analysis. All media were prepared following the instruction of the manufacturers.

2.6. Aerobic Mesophilic Bacterial Count (AMBC). For all microbial load determinations, the standard plate count method was used [22, 23]. Briefly, for both shell rinsate and whole egg content of each sample, 0.1 ml aliquots of appropriate serial dilutions (10^{-5} and 10^{-7}) were aseptically transferred into respectively labeled separate plates of plate count agar (PCA, Difco) and spread plated with the sterile bent glass rod (glass rod was sterilized by dipping into ethanol and burning off the alcohol). The inoculated plates were incubated at 37°C for 48 to 72 hrs. At the end of the incubation, the colonies were counted using Quebec Dark-Field Colony Counter (Richert), and plates having between 30 and 300 colonies were considered to calculate the average.

2.7. Staphylococcus aureus Count (SC). For both shell rinsate and whole egg content of each sample, appropriate dilutions ($10^{-3}$and 10^{-4}) were inoculated by spread plating onto the Mannitol salt agar (MSA) plates as for the AMBC above and incubated at 37°C. At the end of the incubation, plates with countable typical yellow colonies (30 to 300) were considered for calculations.

2.8. Enterobacteriaceae Count (EC). For both shell rinsate and whole egg content of each sample, appropriate dilutions (10^{-5} and 10^{-7}) were inoculated by spread plating as for AMBC above onto violet red bile agar (VRBA) with glucose and lactose (MU1684; HiMedia Labs, India) plates and incubated at 37°C. At the end of the incubation, plates with countable colonies (pink and colorless ones) were considered for calculations.

2.9. Total Coliform Count (TCC). From the same plates considered in EC above, countable pink-red colored colonies only were counted for calculations.

2.10. Fecal Coliform Count (FCC). From the plates used in EC above, countable pink-red colonies surrounded by a zone of acid precipitated bile were considered for calculations.

2.11. Yeast and Mold Count (YAMC). From appropriate tenfold dilution, 0.1 ml aliquots were spread plated onto the surface of potato dextrose agar (PDA, HiMedia, India) supplemented with 1% (w/v) each of chloramphenicol [23, 24]. The plates were then incubated at room temperature (25°C) for one week in a closed box to protect them from dust and insects. Finally, plates with 30 to 300 colonies were considered to estimate the average YAMC of the samples in CFU/ml.

2.12. Determination of Dominant Aerobic Mesophilic Bacteria. From countable plates used in the AMBC above, five to ten distinct colonies were picked separately and purified by repeated subculturing on nutrient agar plates. The purified isolates were maintained in 20% glycerol cryopreservation vials at −20°C until further characterization. Cryopreservation was done by mixing 800 microliters of the broth culture of each isolate with 200 microliters of sterile glycerol [25]. At the end of all sample analyses, the purified and preserved isolates were reactivated to check for purity and viability by streaking on nutrient agar plates and overnight incubation at 37°C. Well-isolated colonies from actively growing plates were characterized by colony morphology, gram staining, and microscopy. The result of gram reaction was used to guide further biochemical tests including catalase test, oxidase test, reaction on triple sugar iron agar (TSI), sulfide indole motility (SIM) medium, and indole test [22, 26]. Briefly, for the catalase test a portion of the well-isolated colony of the test bacterium was mixed with 3% hydrogen peroxide on a clean glass slide. The formation of bubbles indicated a positive test, while the absence showed a negative result [22, 26]. For the oxidase test, a portion of the well-isolated colony was smeared on a filter paper strip impregnated with freshly prepared Kovac's oxidase reagent. The formation of a deep purple color within five to ten seconds constituted a positive oxidase test and the absence of a negative test [22, 26].

The urease test was done on the urea agar slant by taking a portion of the colony of the test bacterium using an inoculating needle and stabbing the butt and streaking the slant. After inoculation, the tube was incubated at 37°C for 24 hours. At the end of the incubation, the tube was examined for color change from yellow to pink that indicates a positive test [22, 26].

TSI Test: using an inoculation needle, a portion of the colony of the test bacterium was taken and stabbed into the center of the TSI agar butt and streaked on the slant. The inoculated tube was then incubated at 37°C for 24 hours. At the end of the incubation, reactions are noted as acid/acid (yellow slant/yellow butt) that indicates fermentation of dextrose, lactose, and/ or sucrose. An alkaline/acid (red slant/yellow butt) indicates the fermentation of dextrose only. An alkaline/alkaline (red slant/red butt) indicates the absence of carbohydrate fermentation. Blackening of the medium occurs in the presence of hydrogen sulfide, and bubbles or cracks in the agar indicated gas production [22, 26].

Likewise, the SIM medium was inoculated by taking a portion of the colony of the test bacterium using an inoculating needle and stabbing the center. The inoculated tube was incubated at 37°C for 24 hours and examined. Blackening of the medium and growth away from the stab line indicated hydrogen sulfide production motility, respectively. To determine the indole production, two to three drops of Kovac's reagent were added and the appearance of red ring indicated a positive test. The selected battery of biochemical test allowed putative identification of the isolates to the generic level [22, 26].

2.13. Determination of the Proportion of Escherichia coli in the FCC. From countable plates used in FCC above, five to ten typical colonies (pink-red colonies with bile precipitate) were picked and purified by repeated subculturing. The purified isolates were streaked onto plates of eosin methylene blue agar (EMBA) and also subjected to the indole-methyl red-Voges Proskauer-citrate (IMVC) biochemical test [22, 26]. Isolates that showed black colonies with green metallic sheen on EMB agar and positive for indole and methyl red test but negative for Voges Proskauer and citrate utilization test were identified as *E. coli.*

2.14. Detection of Salmonella Species. For both shell rinsate and whole egg content of each sample, a loop full of the shell surface rinsate and whole egg homogenate sample was directly streaked separately onto the surface of Salmonella Shigella Agar (SSA, Oxoid). To resuscitate stressed cells, streaking on the same media was also done after overnight culture of 0.1 ml aliquots from 1 : 10 dilution into a tube containing 5 ml of sterile nutrient broth. All plates were incubated at 37°C for 48 h, and at the end of the incubation, typical colonies (black) were picked and purified by repeated subculturing. Purified presumptive salmonellae isolates were subjected to selected biochemical tests for confirmation by inoculation into sulfide indole motility (SIM) agar, triple sugar iron (TSI), and urea agar. Isolates that were Gram-negative small rods, non-lactose fermenters, hydrogen sulfide-positive, indole negative, motile, and urease-negative were putatively identified as Salmonella species [22, 26].

2.15. Data Analysis. All enumerations were done in duplicates, and values were transformed into $\log_{10}$ unit for ease of manipulation. To calculate the average load from multiple plates, the following formulae were used [27]:

$$N = \frac{\text{Sum of colonies from all countable plates}}{(N_1 + 0.1N_2)D}, \quad (1)$$

where N_1 is the number of plates with countable colonies in the first dilutions, N_2 is the number of plates with countable colonies in the second dilution, and D is the dilution factor corresponding to the first dilution.

The SPSS 20 was used to analyze the data, and ANOVA was used to compare the mean microbial load values for all parameters. The average microbial loads of the egg samples from the different locations were compared, and p values less than 0.05 were used to adjudge the statistical significance.

Table 2: Aerobic mesophilic bacterial count (AMBC) in $\log_{10}$ CFU/ml of the shell surface rinsates and contents of egg samples in Hawassa City, southern Ethiopia, 2019.

Sampling locations	Shell surface rinsate			Internal egg content		
	Mean ± SE	Min	Max	Mean ± SE	Min	Max
Menahariya	9.70 ± 0.22^{a}	8	10.1	9.93 ± 0.19^{ab}	8.6	10.4
Wukro	9.84 ± 0.05^{a}	9.6	10	9.79 ± 0.24^{ab}	8	10.4
Atote	9.57 ± 0.22^{a}	8.1	10.6	9.51 ± 0.10^{b}	8.8	9.8
Addis Ketema	9.03 ± 0.33^{a}	7.9	10.1	7.31 ± 1.40^{b}	ND	10
Bahil Adarash	9.42 ± 0.24^{a}	7.6	10.1	9.59 ± 0.17^{ab}	8.3	10
HUPF	9.73 ± 0.06^{a}	9.5	10.1	9.71 ± 0.20^{ab}	8.3	10.3
Grand total	**9.55 ± 0.09**	**7.6**	**10.6**	**9.31 ± 0.26**	**ND**	**10.4**

HUPF = Hawassa University Poultry Farm; SE = standard error of the mean, min = minimum, max = maximum, ND = not detectable. Means followed by similar superscript letters in the columns indicate that the observed differences in mean microbial loads of the egg samples from the different locations were not statistically significant by Tukey's multiple comparison, at p value <0.05.

3. Results and Discussion

3.1. The Microbial Loads of the Egg Samples

3.1.1. The Aerobic Mesophilic Bacterial Count (AMBC) of the Egg Samples. The overall average AMBC on the surfaces of the shells of the egg samples was 9.55 $\log_{10}$ CFU/ml of rinsate, while that of the egg contents was 9.31 $\log_{10}$ CFU/ml (Table 2). The observed differences in the mean AMBC of the shell rinsates among the egg samples collected from the different locations in Hawassa were not statistically significant ($p > 0.05$). All of the 60 egg samples (100%) in this study showed AMBC greater than 7 $\log_{10}$ CFU/ml of shell rinsate (Table 3). On the other hand, the mean AMBC of the egg contents of the egg samples from Menahariya sub-city was significantly higher than that of samples from Addis Ketema sub-city (7.31 $\log_{10}$ CFU/ml) and Atote ($p < 0.05$). The observed differences in the mean AMBC of the egg contents among samples from all other study sites were not statistically significant (Table 2).

The overall mean AMBC on the eggshells in this study was higher than that of Chaemsanit et al. [28] who reported a

Table 3: Average microbial loads in $\log_{10}$ CFU/ml of the shell surface rinsate of chicken egg samples collected from six different locations in Hawassa City, Ethiopia, 2019.

Sampling locations	AMBC	EC	TCC	FCC	SC	YMC
Menahariya sub-city ($N = 9$)	10	8.6	ND	ND	9.9	9.2
	9.8	7.26	7.7	ND	7.6	9.7
	8	9	9.4	ND	7.5	9.6
	10.1	ND	ND	ND	7.7	9.1
	9.8	8.7	ND	ND	8.1	8.2
	10	6.5	7.5	ND	7.5	9.7
	10	ND	ND	ND	ND	9.6
	9.7	7	7.5	7.5	7.8	7.5
	9.9	6.6	ND	ND	8.4	7.8
	9.8	7	ND	ND	9.1	9.7
Wukro sub-city ($N = 9$)	10	8.6	7.7	ND	9.3	9.6
	10	8.9	9.5	ND	7.7	9.1
	9.8	8.7	ND	ND	8	7.6
	9.9	6.8	7.5	ND	7.5	9.6
	9.7	ND	ND	ND	9.6	9.6
	9.6	7	7.6	7.5	9.2	7.5
	10	8.6	9.1	ND	9.7	9.1
	9.8	ND	ND	ND	ND	9.2
	9.9	7.2	ND	ND	7.4	7.5
Atote sub-city ($N = 9$)	9.5	7.2	ND	ND	7.6	ND
	9.9	7	ND	ND	7.4	6.7
	10.6	7	ND	ND	7.6	ND
	9.5	7.2	ND	ND	7.4	ND
	9.6	8.6	ND	9.5	7.9	7.5
	9.5	5	5.6	ND	9.2	5.7
	8.1	7.3	ND	ND	8	7.2
	9.5	6.6	ND	ND	7.9	7.2
	9.4	8.8	9.7	ND	9.2	5.6
Addis Ketema sub-city ($N = 9$)	8.1	8.8	9.3	7.1	8.4	5.7
	7.9	4.7	ND	ND	7.8	7.2
	10	ND	ND	ND	7.6	5.4
	9.9	8.5	6	9	ND	5.4
	8	8	8.7	ND	7.4	5.5
	10.1	7.2	5.7	ND	ND	7.1
	8.1	ND	ND	ND	8.4	7.2
	9.8	6.8	ND	ND	7.5	7.5
	10.1	9.3	ND	ND	9.2	7.3
Bahil Adarash (Piassa) sub-city ($N = 9$)	9.5	ND	ND	ND	9.1	7.7
	9.8	4.8	ND	ND	9.2	5.5
	9.5	4.6	ND	ND	ND	ND
	9.5	6.9	ND	7.5	9.1	5.8
	9.4	5	5.6	ND	9.1	5.7
	7.6	7.2	ND	ND	9.6	7.3
	9.4	6.6	ND	ND	7.8	7.3
	10	5.4	ND	ND	9.5	7.3
Hawassa University Poultry Farm ($N = 15$)	9.5	ND	ND	ND	9.2	7.8
	9.8	5.7	ND	ND	9.2	5.5
	9.6	5.6	ND	ND	ND	ND
	9.5	7.2	ND	5.6	9.2	5.8
	9.8	7.5	5	ND	7	ND
	10.2	7.3	7.3	5.9	9.3	5.6
	9.7	7.4	ND	ND	9.6	5.5
	10.09	7.7	7.3	ND	9.8	ND
	9.6	7.9	ND	ND	9.3	ND
	9.7	7.2	ND	7.2	7.7	ND
	9.9	5.5	ND	5.5	7.8	ND
	9.6	7.6	ND	7.9	7.9	5.5
	9.9	7.6	ND	ND	7.8	ND
	10	7	ND	5.8	9.4	5.5
	9.9	7.7	7.8	ND	9.4	ND

AMBC = aerobic mesophilic bacterial count, EC = Enterobacteriaceae count, TCC = total coliform count, FCC = fecal coliform count, SC = staphylococcal count, YMC = yeast and mold count, ND = not detected.

Table 4: Mean microbial loads in $\log_{10}$ CFU/ml of the contents of chicken egg samples collected from six different locations in Hawassa City, southern Ethiopia, 2019.

Sampling locations	AMBC	EC	TCC	FCC	SC	YMC
Menahariya sub-city ($N = 9$)	8.6	ND	ND	ND	9.9	9.6
	9.7	8.7	ND	ND	7.6	9.8
	10.2	6.5	ND	ND	7.5	9.9
	10.3	ND	ND	ND	7.7	9.6
	10.2	ND	ND	ND	8.1	7.7
	9.7	9	9.99	ND	7.5	9.6
	10.3	ND	ND	ND	9.6	9.2
	10.4	8.5	ND	ND	9.2	9.1
	10	8.5	ND	ND	9.8	9.6
	9.5	ND	ND	ND	7.7	9.5
Wukro sub-city ($N = 9$)	10	8.8	8.05	ND	7.6	9.7
	10.2	ND	ND	ND	7.7	9.7
	10.2	ND	ND	ND	8	9.6
	9.9	ND	ND	ND	7.5	9.4
	10	9.3	9.95	ND	9.6	9.6
	10.4	8.6	ND	ND	9.2	9.5
	9.9	ND	ND	ND	ND	7.1
	9.8	ND	ND	ND	7.4	ND
Atote sub-city ($N = 9$)	9.5	4.5	5.52	ND	7.6	ND
	9.4	7	ND	ND	7.4	ND
	9.7	ND	ND	ND	7.6	ND
	9.4	4.6	5.65	ND	7.4	ND
	9.8	8.9	9.94	ND	7.9	ND
	9.7	4.6	ND	5.5	9.2	7.7
	9.5	5	ND	5.8	8	9.2
	8.8	7.2	ND	ND	7.9	9.2
Addis Ketema sub-city, ($N = 9$)	9.5	ND	ND	ND	9.2	9.5
	8	ND	ND	ND	8.4	9.3
	9.1	8.6	9.6	ND	7.8	9.3
	9.8	9.1	9.3	9.8	7.6	7.6
	9.6	6.9	ND	ND	10.4	7.6
	ND	8.7	9.18	5.6	10.1	7.5
	ND	6.6	ND	ND	ND	5.5
	10	8.6	5.72	9.6	8.4	5.5
	9.8	9.1	ND	ND	7.6	5.5
	9.6	8.6	ND	9.6	9.2	5.7
Bahil Adarash (Piassa) sub-city ($N = 9$)	8.3	ND	ND	ND	7.9	ND
	9.5	4.9	ND	5.5	9.2	5.6
	9.8	6.9	ND	ND	ND	5.5
	10	5	ND	5.6	9.1	5.5
	9.6	4.6	ND	5.6	9.1	5.5
	9.6	5.1	ND	5.8	9.1	ND
	10	6.9	ND	ND	7.8	ND
	9.9	6.9	ND	7.8	9.5	9.3
Hawassa University Poultry Farm ($N = 15$)	8.3	ND	ND	ND	ND	ND
	9.5	5.2	5.6	5.6	7.5	ND
	9.8	7	5.56	ND	ND	ND
	10	5.1	5.57	5.5	7.5	ND
	9.6	6.6	ND	ND	7.5	ND
	10.1	6.6	ND	ND	7.6	ND
	9.9	4	ND	ND	7.6	ND
	10.3	ND	ND	ND	7.5	ND
	9.9	ND	ND	ND	ND	ND
	9.6	ND	ND	ND	ND	ND
	10.3	ND	ND	ND	ND	ND
	10.3	ND	ND	ND	ND	ND
	8.3	6	ND	ND	7.5	ND
	9.5	6.6	ND	ND	10.1	ND
	9.8	6.8	ND	ND	7.6	ND
	8	6.9	6.03	ND	9.7	7.8

AMBC = aerobic mesophilic bacterial count, EC = Enterobacteriaceae count, TCC = total coliform count, FCC = fecal coliform count, SC = staphylococcal count, YMC = yeast and mold count, ND = not detected.

mean AMBC ranging between 2.9 and 6.2 log CFU/ml of shell rinse for 16 table egg samples from the retail market. The mean AMBC in this study was also higher than 7.2–8 log CFU/ml of shell rinse for samples from poultry farms in Asia reported in the same study. In general, the high number of varying types of microorganisms can be expected on untreated surfaces of raw chicken egg. The number and types of mesophilic aerobic bacteria on the shell surface suggest the manner of handling history and hygienic status of its production environment. Moreover, it allows one to predict the types of microorganisms to be expected in the processed ready-to-consume food that results from it later in its history [3, 14].

Contamination of the internal contents of egg by bacteria via transovarian, oviductal, and trans-shell migration is a widely known phenomenon [29]. With regard to food quality and safety standards, AMBC is a general criterion and indicator of sanitary quality and the extent to which the producers adhered to good agricultural and hygienic practices [30]. Mesophilic aerobic bacteria should not be recovered from any number of samples of whole egg contents in a number exceeding 6 $\log_{10}$ CFU/ml [22, 30]. Even the mean values of the contents of the egg samples from Addis Ketema sub-city (which were the lowest) in this study were more than one log unit higher than the above-recommended limit. Such a high level of AMBC is suggestive of poor microbial quality and incipient spoilage, probably the result of poor hygienic handling, absence of refrigerated storage, and long age of the eggs.

Of the total 60 egg samples, 58 (96.7%) showed mean AMBC greater or equal to 8 $\log_{10}$ CFU/ml of egg content, a value much higher than the recommended standard (Table 4). Chaemsanit et al. [28] reported from a study of 16 egg samples in Asia that only one egg sample (6.25%) showed AMBC at the level of 3 $\log_{10}$ CFU/ml of content, which is much lower than that of this study. A similar study done in Brazil on a total number of 30 egg samples reported a mean AMBC of 6.1 $\log_{10}$ CFU/ml of content [31], which is also lower than that of this study. According to the recommended standard, aerobic mesophilic bacteria must not be recovered from raw liquid egg products in number exceeding 6 $\log_{10}$ units [22, 30]. In this study, 96% of the contents of the egg samples showed mean AMBC that exceeded this limit. The mean AMBC in this study is closer to that of the Nationwide Microbiological Baseline Data Collection Program Survey in the United States for raw liquid eggs that reported a mean AMBC of 8.6 $\log_{10}$ CFU/ml [3].

In a study of pooled contents of 30 egg samples in Eastern Ethiopia, Senbeta et al. [11] reported a mean AMBC of 1.226 $\log_{10}$ CFU/ml for samples from Haramaya University Poultry Farm, 5.378 log CFU/ml for sample from Haramaya Town, 5.596 $\log_{10}$ CFU/ml for samples from Harar City, and 5.597 $\log_{10}$ CFU/ml for samples from Dire Dawa City. These values are much lower than that in this study. The observed differences may be due to variations in the methods used, the age of the egg samples, and hygienic handling. Several factors may account for the contamination of the internal contents of chicken egg. Microorganisms could reach the egg content because of prolonged storage time, favorable extrinsic factors or storage environment, and inappropriate handling and transportation of eggs [32].

3.1.2. The Staphylococcal Count (SC) of the Egg Samples. The overall average staphylococcal count (SC) on the shell surface rinsate of the egg samples was 7.64 $\log_{10}$ CFU/ml of rinsate and that of the internal contents was 7.26 $\log_{10}$ CFU/ml (Table 5). Of the 60 egg samples, only 54 (90%) showed the growth of staphylococci on Mannitol salt agar (MSA) from their shell surface rinsates (Table 3). The observed differences in the mean SC of the shell rinsate among the egg samples collected from all locations were not statistically significant. On the other hand, the contents of only 45 (75%) of the egg samples showed growth of countable staphylococcal colonies on MSA (Table 4). The mean SC of the contents of the egg samples from HUPF (4.20 $\log_{10}$ CFU/ml) was significantly lower than that of samples from Menahariya sub-city (Table 5). The observed differences in the mean SC of contents among the egg samples from all other study sites were not statistically significant.

The natural habitat of staphylococci is the human and animal skin, especially around body orifices [33]. Therefore, they can be expected at varying numbers on non-heat-treated foods of animal origin [14]. Staphylococci also are etiological agents for different poultry diseases such as chronic conjunctivitis in young chickens, inflammation of the navel and gall bladder, blue wing disease, and green liver osteomyelitis complex [34]. Therefore, eggshell contamination by different microorganisms including *Staphylococcus* species may result from the environment, laying hen or handlers. Starting from primary production in the typical backyard poultry farm in the rural Ethiopia, contamination is likely to build up further as eggs are transported over long distance and passed between traders and consumers. Both eggs and live birds are transported either on foot or using public transportation along with other bags, sacks of grains, bundles of firewood, etc. In general, there are no packaging and weight standardization of market eggs and traditional storage methods can lead to deterioration of the quality of table eggs.

The incidence of staphylococci on the eggshell surface rinsates in this study (90%) was higher than that of the reported 68% and 76.8% for egg samples from a large poultry farm (battery cage system) and supermarket retail outlet in Poland [35]. The work done in Poland also reported an incidence of 96.8% from small-scale poultry farm (litter system), which is higher than the incidence in this study. The largest poultry production system in Ethiopia is the backyard and free-range production system, more or less similar to the litter system [36]. Circumstantial observations in the retail market show that eggs are stored at ambient temperature under the humid atmospheric condition that favors the proliferation of contaminating microorganisms and penetration into the contents. Refrigerators are beyond the capacity of low-income communities in developing countries [37]. The egg chain from the private smallholder system in Ethiopia is very tortuous and likely to result in heavy

Table 5: Staphylococcal count (SC) in $\log_{10}$ CFU/ml of the shell surface rinsates and contents of egg samples in Hawassa City, southern Ethiopia, 2019.

Sampling locations	Shell surface rinsate			Internal egg content		
	Mean ± SE	Min	Max	Mean ± SE	Min	Max
Menahariya	7.17 ± 0.93^{a}	ND	9.9	$8.54 \pm .35^{a}$	7.5	9.9
Wukro	7.79 ± 1.01^{a}	ND	9.7	7.44 ± 0.98^{a}	ND	9.7
Atote	7.82 ± 0.19^{a}	7.4	9.2	$7.82 \pm .19^{a}$	7.4	9.2
Addis Ketema	6.26 ± 1.20^{a}	ND	9.2	7.72 ± 1.02^{a}	ND	10.4
Bahil Adarash	8.07 ± 1.02^{a}	ND	9.6	7.88 ± 1.00^{a}	ND	9.5
HUPF	8.76 ± 0.33^{a}	7	9.8	4.18 ± 1.32^{b}	ND	7.6
Grand total	**7.64** ± 0.35	ND	**9.9**	**7.26** ± 0.40	ND	**10.4**

HUPF = Hawassa University Poultry Farm; SE = standard error of the mean, min = minimum, max = maximum, ND = not detectable. Means followed by similar superscript letters in the columns indicate that the observed differences in mean microbial loads of the egg samples from the different locations were not statistically significant by Tukey's multiple comparison, at p value <0.05.

contamination that also increases the risk of penetration of the internal contents of the eggs [19].

The overall mean staphylococcal count (SC) in this study (7.31 log unit) was much higher than 4.4 $\log_{10}$ CFU/ml contents of egg samples from Brazil [31] and 3.2 $\log_{10}$ CFU/ml reported for contents of 10 egg samples from Mauritius [10]. In a study of 25 chicken egg samples from a farm in Egypt, Awny et al. [38] reported a mean SC of $4.0 \times 10^2 \pm 1.08 \times 10^2$ CFU/ml of contents, which is much lower than this study. The high level of staphylococcal count in this study reflects the excessive handling history between laying and prolonged storage in the retail market. As stated in the foregoing sections, staphylococci are part of the human and animal normal flora and once transferred to the egg surface may find their way into the egg contents as in the case of other bacteria. In a study of 375 egg samples consisting of 125 from each large poultry farm, small-scale poultry farm, and retail supermarket in Poland, Stepiens-Psyniak et al. [35] reported the detection of both coagulase-positive and coagulase-negative staphylococci from egg white (13 or 3.5%) and yolk (199 or 53.1%) of the samples. The incidence of staphylococci in the egg contents (51 of 60 or 85%) of the samples in this study was much higher than the report from Poland. How nonmotile Gram-positive bacteria like Staphylococcus liable to lysozyme can penetrate and survive in the unfavorable internal contents of eggs may seem enigmatic. It has been shown that when the temperature of eggs is cooled after oviposition, the internal contents undergo contraction, which creates a negative pressure and effectively pulls all the surface bacteria across the shell into the contents. The harsh environment of the egg white may retard the growth and further penetration of the contaminating microbes, but once they reach the yolk, it provides a much favorable environment for multiplication [29].

According to the US Compendium of Microbiological Criteria for Food, [3] the number of pathogens (*Staphylococcus aureus* and other coagulase-positive staphylococci) above 5 log CFU/ml in row food commodities is considered as potentially hazardous. A sufficient amount of enterotoxin would be produced at this level of SC. Staphylococcus enterotoxin is heat-stable; therefore, reprocessing is not recommended [39]. This study did not include confirmation with coagulase test or determination of enterotoxin production, but the mean SC level in the internal contents of the egg samples from the small-scale vendors was in excess of 7 log units while that in samples from the HUPF was between 4 and 5 log units (Table 6). The most common causes of such a high level of *Staphylococcus aureus* count are inadequate washing, lack of refrigeration, and poor hygienic handling practices. In addition, mixing dirty or cracked egg with sound ones in a storage basket can lead to contamination and growth of microbes in the row eggs [31].

3.1.3. The Enterobacteriaceae Count (EC) of the Egg Samples. The overall average *Enterobacteriaceae* count (EC) of the shell surface rinsates of the egg samples in this study was 6.13 $\log_{10}$ CFU/ml of shell rinsate and that of the internal contents was 4.52 $\log_{10}$ CFU/ml (Table 7). Of the total 60 egg samples, eight (13.33%) of them did not show detectable growth of *Enterobacteriaceae* in the eggshell rinsates (Table 3). The observed difference in the mean ECs of shell rinsate among the egg samples collected from all sites was not statistically significant ($p > 0.05$). On the other hand, with regard to the internal contents 20 (33.33%) of the egg samples showed no detectable *Enterobacteriaceae* (Table 4). The observed difference in the mean ECs of the egg contents among the egg samples collected from all sites was not statistically significant ($p > 0.05$).

The overall mean EC of the shell rinsates of the egg samples in this study (6.13 log units) was much higher than 1.5 $\log_{10}$ CFU/ml of rinsate reported by Bahobail et al. [40] for 175 egg samples from Saudi Arabia. Based on analysis of white shell and brown shell egg samples from markets in Egypt, Al-Ashmawy et al. [41] reported the detection and counting of *Enterobacteriaceae* in 52% of both types of egg samples at a mean EC level of 6.3 log units. This value is closer to, but slightly higher than that of egg samples in this study. Only 20% of both types of the egg samples from the Egyptian markets were reported to have mean ECs that exceeded the European Union Council recommended maximum level of 2 log units [42]. In this study, *Enterobacteriaceae* were detected and counted in the shell surface rinsates of 52 of 60 (86.7%) of the egg samples and all the positive samples had EC levels of more than 4 log units (Table 3). The high count on the eggshell surface reflects gross defect in good agricultural and hygienic practices in the farm and unhygienic handling after laying, transportation, and storage in the retail markets. A higher load of contamination on the shell and improper storage conditions (humidity) enhance trans-shell migration and contamination of internal contents and lower the shelf life of eggs.

In a study done on fresh egg samples obtained from the poultry farm in Nigeria, Folorunsho and Charles [43] reported a mean EC of 2.25 $\log_{10}$ CFU/ml of contents, which is much lower than that of samples in this study. As stated earlier, *Enterobacteriaceae* are general indicators of hygiene and good agricultural practice. The maximum recommended level of *Enterobacteriaceae* count (EC) in egg products by the

Table 6: Total coliform count (TCC) in $\log_{10}$ CFU/ml of the shell surface rinsates and contents of egg samples in Hawassa City, southern Ethiopia, 2019.

Sampling locations	Shell surface rinsate			Egg content		
	Mean ± SE	Min	Max	Mean ± SE	Min	Max
Menahariya	3.57 ± 1.42^a	ND	9.4	1.11 ± 1.11^a	ND	9.99
Wukro	4.6 ± 1.47^a	ND	9.5	2.67 ± 1.37^a	ND	9.95
Atote	0.62 ± 0.62^a	ND	5.6	2.35 ± 1.25^a	ND	9.94
Addis Ketema	4.38 ± 1.45^a	ND	9.7	3.76 ± 1.53^a	ND	9.6
Bahil Adarash	0.62 ± 0.62^a	ND	5.6	0.00 ± 0.00^a	ND	0
HUPF	1.37 ± 0.92^a	ND	7.3	1.24 ± 0.82^a	ND	5.6
Grand total	**2.53** ± 0.05	ND	**9.7**	**1.85** ± 0.47	ND	**9.99**

HUPF = Hawassa University Poultry Farm; SE = standard error of the mean, min = minimum, max = maximum, ND = not detectable. Means followed by similar superscript letters in the columns indicate that the observed differences in mean microbial loads of the egg samples from the different locations were not statistically significant by Tukey's multiple comparison, at p value <0.05.

Table 7: *Enterobacteriaceae* count (EC) in $\log_{10}$ CFU/ml of the shell surface rinsates and contents of egg samples in Hawassa City, southern Ethiopia, 2019.

Sampling locations	Shell surface rinsate			Internal egg content		
	Mean ± SE	Min	Max	Mean ± SE	Min	Max
Menahariya	5.96 ± 1.17^a	ND	9	4.58 ± 1.47^a	ND	9
Wukro	6.18 ± 1.20^a	ND	8.9	3.73 ± 1.49^a	ND	9.3
Atote	7.01 ± 0.31^a	5	8.6	4.64 ± 1.01^a	ND	8.9
Addis Ketema	5.87 ± 1.19^a	ND	8.8	6.40 ± 1.25^a	ND	9.1
Bahil Adarash	5.53 ± 0.85^a	ND	9.3	5.43 ± 0.81^a	ND	8.6
HUPF	6.23 ± 0.83^a	ND	7.9	2.32 ± 0.94^a	ND	6.6
Grand total	**6.13 ± 0.38**	ND	**9.3**	**4.52**	ND	**9.3**

HUPF = Hawassa University Poultry Farm; SE = standard error of the mean, min = minimum, max = maximum, ND = not detectable. Means followed by similar superscript letters in the columns indicate that the observed differences in mean microbial loads of the egg samples from the different locations were not statistically significant by Tukey's multiple comparison, at p value <0.05.

European Commission Directive is 2 log units [42]. Their detection at mean levels greater than 4 log units in this study suggested poor sanitation and unhygienic practice that led to cross-contamination and/or prolonged storage time between laying in the farm and the retail market that facilitated trans-shell migration and buildup. A progressive increase in the microbial load of both shell and internal contents of chicken egg has been demonstrated even under refrigerated storage [21]. Although they are generally heat-labile and destroyed by regular cooking temperature, the consumption of undercooked or raw egg products may expose them to pathogenic members such as salmonellae. Drinking egg as a traditional medication for respiratory and other ailments is a widespread practice in different parts of Ethiopia.

3.1.4. The Total Coliform Count (TCC) of the Egg Samples. The overall average TCC in the surface shell rinsates and contents of the egg samples was 2.53 $\log_{10}$ CFU/ml and 1.87 $\log_{10}$ CFU/ml, respectively (Table 6). Of the 60 samples, only 19 (31.67%) showed countable number of coliform bacteria in the shell surface rinsate (Table 3). Likewise, 46 (76.67%) of the egg samples showed no detectable coliform bacteria in their contents (Table 4). The observed difference in the mean TCC of both shell rinsate and egg contents among the egg sample collected from all locations was not statistically significant (Table 6).

The coliform bacteria are a functional subgroup of the family *Enterobacteriaceae* that ferment lactose with acid and gas production within 48 hrs at mesophilic temperature [44]. The classical members include *Escherichia* coli, *Enterobacter* species, *Klebsiella* species, and *Citrobacter* species. With regard to safety and quality, TCC is a general hygienic indicator and a high level of coliform counts indicates unsanitary conditions or poor hygienic practices during or after food production. Because some members are present as normal inhabitants of environments such as soil, vegetation, and water, their presence in food samples does not necessarily indicate fecal contamination or presence of enteric pathogens [45]. The mean TCC on shells of the egg samples in this study (2.53 log units) was less than the findings of Periera et al. [46] who reported 4 $\log_{10}$ CFU/ml of shell rinsate for egg samples collected from different commercial establishments and conditions of storage in Brazil. Although most members of coliform bacteria are considered harmless, several pathovars of *E. coli* have emerged as important foodborne pathogens [47]. Heavy contamination of shell surface may lead to contaminate the internal contents *via* trans-shell migration or cross-contamination during breakage [29].

The overall mean TCC of the contents of the egg samples in this study (1.85 log unit) was less than the maximum acceptable standard limit of 3 log CFU/ml [30]. In a similar study done in Brooklyn New York, Yaratha et al. [48] reported a mean TCC of 3.4 log of CFU/ml contents of egg samples, which is above the maximum acceptable standard limit. The overall mean TCC of the contents of the egg samples in this study was higher than 1.23 $\log_{10}$ CFU/ml of pooled contents of 30 egg samples from Haramaya University Poultry Farm [11]. In the same study, Senbata and coworkers [11] reported a mean TCC of 5.3 $\log_{10}$ CFU/ml for the contents of egg samples from Haramaya Town, 5.51 $\log_{10}$ CFU/ml for samples from Harar City, and 5.49 $\log_{10}$ CFU/ml for samples from Dire Dawa City—all located in Eastern Ethiopia. The above three values are much higher than this study.

The differences in the TCC levels of egg samples among the different studies could be due to true differences in the implementation of good agricultural and hygienic practices or difference in the methods used in the analysis. As stated in the foregoing sections, coliform bacteria are general indicators of hygienic and sanitary conditions and members of the coliform bacteria are generally harmless. However, the potential presence of foodborne pathogenic *E. coli* should be borne in mind and the drinking/consumption of raw or undercooked eggs should be discouraged.

3.1.5. The Fecal Coliform Count (FCC) of the Egg Samples. The overall average fecal coliform count (FCC) in the shell surface rinsates and contents of the egg sample was 1.23

$\log_{10}$ CFU/ml and 1.62 $\log_{10}$ CFU/ml, respectively (Table 8). Of the 60 samples, only 11 egg samples (18.33%) showed detectable fecal coliform bacteria in the eggshell surface rinsates (Table 3). Likewise, only 14 (23.33%) of the egg samples showed the detectable and countable fecal coliform bacteria in their contents (Table 4). The observed differences in the mean FCC of the shell rinsates of the egg samples among the different locations were not statistically significant. On the other hand, egg samples from Menahariya and Wukro sub-cities showed no detectable fecal coliform bacteria in their contents (Table 8). The mean FCC of the contents of the egg samples from Bahil Adarash was significantly higher than those of Menahariya and Wukro sub-city (Table 8).

In a similar study done in Beni Suef City, Egypt, on 170 egg samples, El-Kholy et al. [20] reported the average FCC to be 1.99 $\log_{10}$ CFU/ml of eggshell rinsates. This value is higher than the overall average fecal coliform count (1.23 log units) of the egg samples in this study. Based on the investigation of eggshell surface in three US processing plants, Musgrove et al. [29] reported FCC (*E. coli*) ranging between 0.62 and 0.9 $\log_{10}$ CFU/ml of rinse for egg samples. This value is much lower than this study. The higher level of the FCC bacteria in this study indicates poor implementation of good agricultural and hygienic practices at the primary production level and subsequent handling and storage in the egg chain. A high level of FCC of the egg samples signals a potential hazardous level presence of enteric pathogens that may lead to the contamination of internal content *via* trans-shell penetration or cross-contamination during breakage.

In similar studies done on 30 egg samples in Beni Suef City, Egypt, El-Kholy et al. [20] reported a mean FCC of 0.45 log CFU/ml of contents, which is lower than the overall average FCC finding of this study. As stated previously, FCC is used as an indicator of hygiene measures and the level of fecal contamination and the possible presence of enteric pathogens [3]. The higher level of FCC in the egg contents as compared to the shell surface in this study suggested growth from an initial contamination *via* trans-shell migration. This is facilitated by the general absence of refrigerated storage or cold chain during transport from the farm to the market.

3.1.6. The Yeast and Mold Count (YMC) on Eggshell Surface. The overall mean of yeast and mold count (YMC) of the shell surface rinsates and the contents of all the egg samples was 6.26 $\log_{10}$ CFU/ml and 5.49 $\log_{10}$ CFU/ml, respectively (Table 9). Of the 60 egg samples, only 48 (80%) showed countable fungal growth in the shell rinsate (Table 3). Likewise, fungal growth was detected and counted for the contents of only 39 (65%) egg samples, while the contents of 21 egg samples (35%) showed no growth (Table 4). The mean YMC of the surface shell rinsates of the egg samples from Wukro sub-city and Menahariya sub-city was significantly higher than those of HUPF and Atote (Table 9). The mean YMC of the contents of the egg samples from Menahariya and Atote sub-cities was significantly higher than those of egg samples from other locations (Table 9). The high YMC of the egg samples from Menahariya and Wukro sub-cities could be because of older age, or prolonged storage of the eggs under favorable conditions for fungal growth [10].

Table 8: Fecal coliform count (FCC) in $\log_{10}$ CFU/ml of the shell surface rinsates and contents of egg samples in Hawassa City, southern Ethiopia, 2019.

Sampling locations	Shell surface rinsate			Egg content		
	Mean ± SE	Min	Max	Mean ± SE	Min	Max
Menahariya	0.83 ± 0.83^{a}	ND	7.5	0.00 ± 0.00^{b}	ND	ND
Wukro	0.83 ± 0.83^{a}	ND	7.5	0.00 ± 0.00^{b}	ND	ND
Atote	1.06 ± 1.06^{a}	ND	9.5	1.26 ± 0.83^{ab}	ND	5.8
Addis Ketema	1.79 ± 1.19^{a}	ND	9	2.78 ± 1.44^{ab}	ND	9.8
Bahil Adarash	0.83 ± 0.83^{a}	ND	7.5	4.43 ± 1.19^{a}	ND	9.6
HUPF	2.03 ± 1.03^{a}	ND	7.2	1.23 ± 0.82^{b}	ND	5.6
Grand total	**1.23** ± 0.38	ND	**9.5**	**1.62** ± 0.41	ND	**9.8**

HUPF = Hawassa University Poultry Farm; SE = standard error of the mean, min = minimum, max = maximum, ND = not detectable. Means followed by similar superscript letters in the columns indicate that the observed differences in mean microbial loads of the egg samples from the different locations were not statistically significant by Tukey's multiple comparison, at p value <0.05.

Table 9: Yeast and mold count (YMC) in $\log_{10}$ CFU/ml of the shell surface rinsates and contents of egg samples in Hawassa City, southern Ethiopia, 2019.

Sampling locations	Shell surface rinsate			Internal egg content		
	Mean ± SE	Min	Max	Mean ± SE	Min	Max
Menahariya	8.93 ± 0.29^{a}	7.5	9.7	9.34 ± 0.22^{a}	7.7	9.9
Wukro	9.0 ± 0.28^{a}	7.5	9.7	9.10 ± 0.32^{a}	7.1	9.7
Atote	4.64 ± 1.18^{bc}	ND	7.5	2.90 ± 1.46^{b}	ND	9.2
Addis Ketema	6.29 ± 0.31^{ab}	5.4	7.5	7.48 ± 0.56^{a}	5.5	9.5
Bahil Adarash	5.99 ± 0.80^{ab}	ND	7.7	4.12 ± 1.11^{b}	ND	9.3
HUPF	2.73 ± 1.10^{c}	ND	7.8	0.00 ± 0.00^{c}	ND	0
Grand total	**6.26** ± 0.43	ND	**9.7**	**5.49** ± 0.56	ND	**9.9**

HUPF = Hawassa University Poultry Farm; SE = standard error of the mean, min = minimum, max = maximum, ND = not detectable. Means followed by similar superscript letters in the columns indicate that the observed differences in mean microbial loads of the egg samples from the different locations were not statistically significant by Tukey's multiple comparison, at p value <0.05.

The overall mean YMC of the shell rinsate of the egg samples in this study (6.26 log units) was higher than 3.5 $\log_{10}$ CFU/ml on eggshell rinsate of 100 egg samples from El-Beheira Governorate, Egypt [49]; 5.4 $\log_{10}$ CFU/ml of shell rinsates for 120 egg samples from Nigeria [50]; the 3.2 $\log_{10}$ CFU/ml reported from Mauritius [10], and the 2.44 $\log_{10}$ CFU/ml reported for 75 egg samples in Alexandria, Egypt [21]. The degree of contamination of eggs depends on hygienic handling starting at the farm [51] and during transportation and distribution to the retail market and handling and storage at the retail level. The higher level of the YAMC in this study reflects the poor hygienic handling of the eggs after laying and prolonged storage at the retail level.

Extrinsic factors such as storage under ambient temperature and high humidity and diffusion of vapour from egg content favor the growth of microscopic fungi on

eggshell [50]. Owing to their ability to withstand extreme environments, fungi can germinate at minimal water activity on eggshell surface and grow to produce a large quantity of spores [10]. Colonization and growth of pathogenic and spoilage fungi on shell surface and penetration upon prolonged storage into the internal contents have been reported before [52, 53]. More recently, Tomczyk et al. [54] reported the finding of mycotoxin type A and type B trichothecenes on the eggshell and egg white samples containing *Fusarium culmorum*.

The overall mean YMC of the contents of the egg samples in this study was higher than that of a similar study done on 75 chicken eggs in Alexandria, Egypt, that reported 1.2 $\log_{10}$ CFU/ml of egg content [21]. In an earlier study, Folorunsho and Charles [43] reported a mean YMC of 2.4 $\log_{10}$ CFU/ml for contents of egg samples from Western Nigeria, which is much smaller than this study. Higher levels of fungi in egg contents as in this study reflect incipient spoilage. The size of the spores of most fungi is small enough to pass through micropores in the eggshell matrix [55]. Once spores find their way into the egg proper, they are more adept in withstanding the harsh environment in the egg white than bacteria to germinate and may elaborate mycotoxins. In a study of 50 egg samples from a backyard farm in India, Rajmani et al. [53] reported the isolation of six fungal genera including mycotoxigenic species.

3.1.7. The Dominant Aerobic Mesophilic Bacterial (AMB) Genera. A total of 209 aerobic mesophilic bacteria (AMB) were isolated by picking and purification of morphologically distinct colonies from plate count agar plates. Of these, 114 were from eggshell rinsates and the remaining 95 from egg contents in the samples. Gram staining microscopy revealed that 140 of 209 isolates (66.98%) were Gram-positive bacteria, whereas 69 isolates (33.01%) were Gram-negative bacteria. Further selected biochemical tests allowed putative identification of the majority of the isolates (174 or 83.25%) into seven bacterial genera, while 35 of the isolates (16.75%) remained unidentified (Figure 1).

The majority of the AMB isolates from the eggshell rinsate (82 of 114 or 71.93%) were Gram-positive bacteria consisting of 28 (24.56%) *Micrococcus*, 22 (19.3%) *Staphylococcus*, 13 (11.4%) *Bacillus* species, and 19 (16.67%) unidentified non-endospore-forming Gram-positive rods (NSGPRs). The remaining 32 isolates from the eggshell rinsate were Gram-negative bacteria dominated by *Pseudomonas* (30 of 114 or 26.32%) with two (1.75%) isolates related to *Enterobacter* (Figure 1). Therefore, overall MAB genera of the eggshell rinsates were dominated by *Pseudomonas* followed by *Micrococcus* and *Staphylococcus*.

Likewise, the MAB isolates from the internal contents of the egg samples (58 of 95 or 61.05%) were also dominated by Gram-positive bacteria consisting of *Micrococcus* (18 of 95 or 18.95%), *Staphylococcus* (17 of 95 or 17.89%), unidentified non-endospore-forming Gram-positive rods (12 of 95 or 12.63%), and *Bacillus* (11 of 95 or 11.28%). The Gram-negative MAB genera of the egg contents were dominated by *Pseudomonas* species (25 of 95 or 26.32%), while *Enterobacter* species (4 of 95 or 4.21%), *Klebsiella* species (2 of 95 or 2.11%), *Shigella* species (2 of 95 or 2.11%), and unidentified members of the family *Enterobacteriaceae* (4 of 95 or 4.21%) constituted the minor group (Figure 1). Interestingly, the same bacterial genera as in the eggshell surface of *Pseudomonas*, *Micrococcus,* and *Staphylococcus* were also predominant in the egg contents (Figure 1).

Surface shell contamination is a common occurrence and indeed is the reason for the existence of regulations in the United States [56] and Sweden [57] that require washing and disinfection of table eggs before they are considered marketable to the public. However, washing would not help eggs inherently contaminated *via* transovarian from an infected hen. In European Union member countries, shell eggs are not washed to avoid removal of the cuticle, which is thought an important barrier that prevents penetration of contaminating microbes into the interior through pores [58]. In all cases, producers are required to follow good agricultural and hygienic practices in the farm and retail markets. There is no such public health regulation or guideline in Ethiopia, and it is therefore quite common to encounter eggs with their shell surface visibly soiled with the excreta of laying hen.

While shell egg is naturally equipped with barriers such as cuticle, shell, and internal membranes, failure in this defense often occurs. Indeed, it is a common knowledge that spoilage and human pathogenic microbes penetrate the interior of the egg contents through pores in the eggshell [53]. The pores are located on the exterior surface of the eggshell. The average egg contains over 7500 pores, most of which are located on the large end of the egg [58]. The pore diameter is large enough to allow fungal spores and most bacteria into the interior, and it has been found that older hens lay eggs with a larger pore size that facilitate microbial penetration [59]. As discussed in the previous sections, when the warm egg cools after oviposition from near the body temperature of the hen (ca 42°C) to that of the environment, the contraction of the internal contents occurs, which leads to a negative suction force that effectively pulls the surface contaminants into the interior via the pores. Therefore, the microflora of the nest and that of the hen that the egg picks in the early hours after laying are critical factors for the type of microbial contamination of the egg [29].

The detection of isolates related to *Shigella* species in egg contents is of great concern since they are highly virulent pathogens with very low infective dose. In a similar study done in Egypt, Al-Ashmawy [41] also reported the detection of *Shigella* species in the contents of one of 25 (4%) duck egg samples. Unlike most foodborne pathogens, Shigellae has no animal reservoir [14] and the source of contamination of eggs could therefore be traced to an infected human handler or carrier. The organism is, however, nonmotile, and how it manages to penetrate the interior is enigmatic. It may be that it happened due to heavy initial contamination and storage under favorable conditions for the survival and growth of the organisms.

Members of the genus *Pseudomonas* are also saprophytic bacteria found in soil and water and associated with a wide range of foods. They are the most common spoilage bacteria

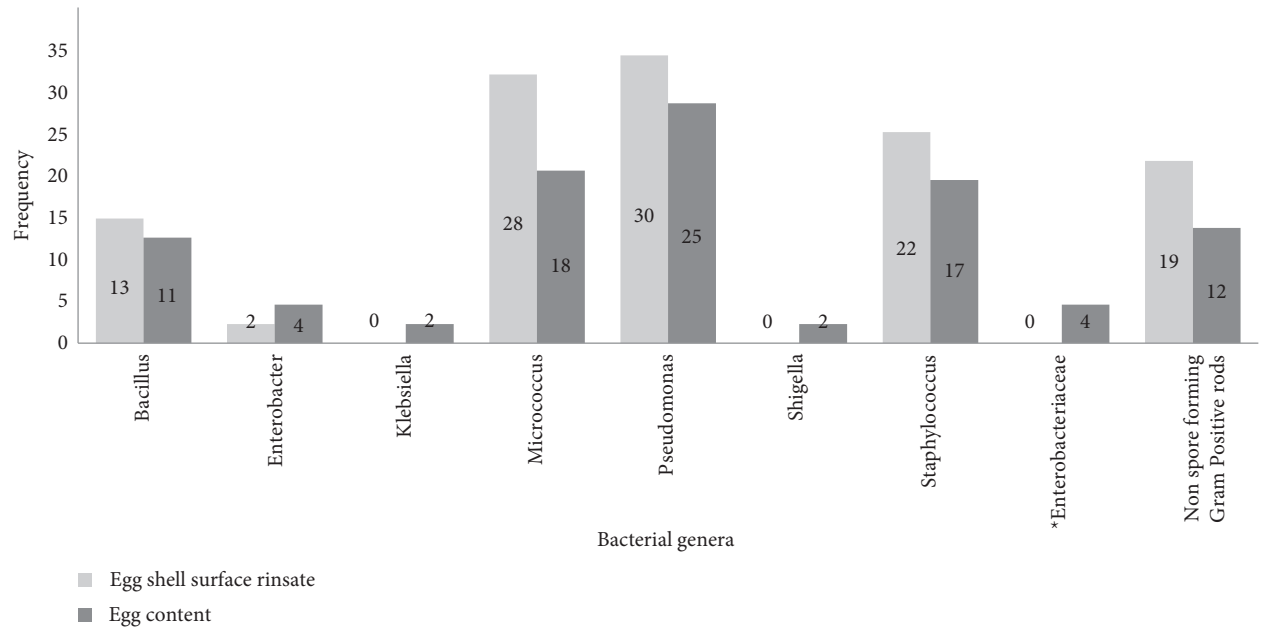

Figure 1: Frequency distribution of the dominant aerobic mesophilic bacterial genera isolated from the shell surface rinsates and contents of egg samples in Hawassa City.

involved in eggs under refrigerated storage conditions [14]. In line with this study, Abdulah [60] also reported that the Gram-positive bacteria including *Micrococcus* and *Staphylococcus* are the predominant bacteria on eggshell. *Micrococci* are normal inhabitants of mammalian skin and can also occur in a wide range of environments including water, soil, dust, animal feed, and hide [14]. Though not spore-forming, *Micrococcus* can survive for an extended period of time at low-temperature, high-salt, low-water activity, and nutrient-deficient conditions [61]. It is therefore not unusual to encounter them on the eggshell and the unfavorable environments of the internal egg content. Although most species are harmless commensals, some can be opportunistic pathogens in people with the compromised immune system [62]. Likewise, Chaemsanit et al. [28] also reported that *Micrococcus* and *Staphylococcus* are among the predominant AMB of chicken eggshells.

3.1.8. The Incidence of Escherichia coli in the Eggshell Rinsate and Egg Contents. Isolates related to *E. coli* were detected in the shell rinsate in only six of the total of 60 (10%) samples (Figure 2). On the other hand, the content of only one egg sample (from Bahil Adarash) was positive for *E. coli* (data not shown).

The incidence of *E. coli* on eggshell in this study (10%) was lower than that of Chaemsanit et al. [28] who reported the detection on 3 of 16 (18.75%) egg samples. In a study of different egg samples consisting of white shell, brown shell, Baladi hen, and duck egg samples from Egyptian markets, Al-Ashmawy [41] reported the detection of E. coli in 4%, 32%, 32%, and 16% of the shell rinsates, respectively. The reported incidence in the contents of the respective types of egg samples was 0%, 4%, 32%, and 4%. As stated earlier, *E. coli* is used as both indicator organism for fecal contamination and index of possible occurrence of enteric pathogens. Therefore, the source of *E. coli* contamination probably is handling persons with poor personal hygiene and environment with poor sanitation.

Right after being laid, eggs have an optimum temperature for the growth of *E. coli*, but during delivery and storage the temperature will fall to ambient and decrease the growth of pathogenic *E. coli* [63]. The incidence of *E. coli* in the egg samples from the Hawassa University Poultry Farm (HUPF) was higher than those of samples from small-scale retailers, which were in line with the report from a similar study by Okorie-kanu et al. [63]. In contrast with this study, Folorunsho and Charles [43] reported the absence of *E. coli* on egg samples at the farm level on both rinsed and unrinsed eggshells. In the same report, it was stated that *E. coli* was found at less than 1 $\log_{10}$ CFU/ml of eggshell rinsate for samples from a supermarket and farm level immediately assessed after laying [10]. The contamination of egg content could also result due to inappropriate disinfection of the eggshell during the analysis of the egg content bacteria while breaking the egg to take a sample [63].

3.1.9. The Incidence of Salmonella in Eggshell Rinsate and Egg Content. Isolates related to *Salmonella* species were detected in the eggshell rinsate and contents of 6 (10%) and 8 (13.8%) of the egg samples (Figure 3). Interestingly, the sources of the positive samples on shell surface and egg content were the same three locations. These were Menahariya, Wukro, and Atote sub-cities. However, only three egg samples from Menahariya sub-city were positive on both their shell and internal content (Figure 3).

Based on the study of 400 egg samples from a poultry farm and open market in Kombolcha Town, Amhara Region of Ethiopia, ASSEFA and coworkers [4] reported the

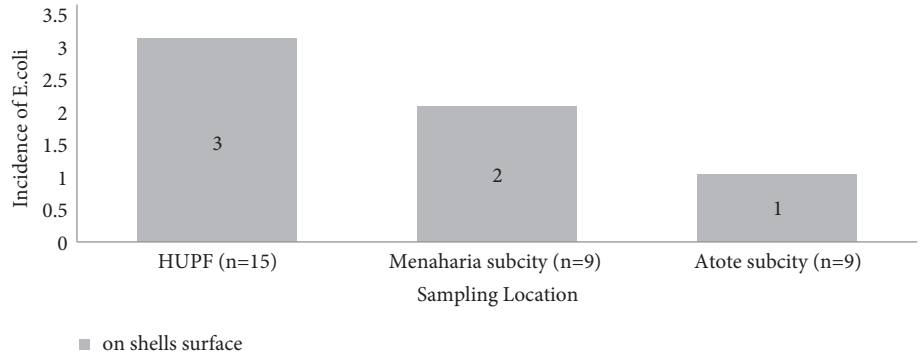

Figure 2: Incidence of E coli in shell surface rinsates of egg samples collected from different markets in Hawassa City.

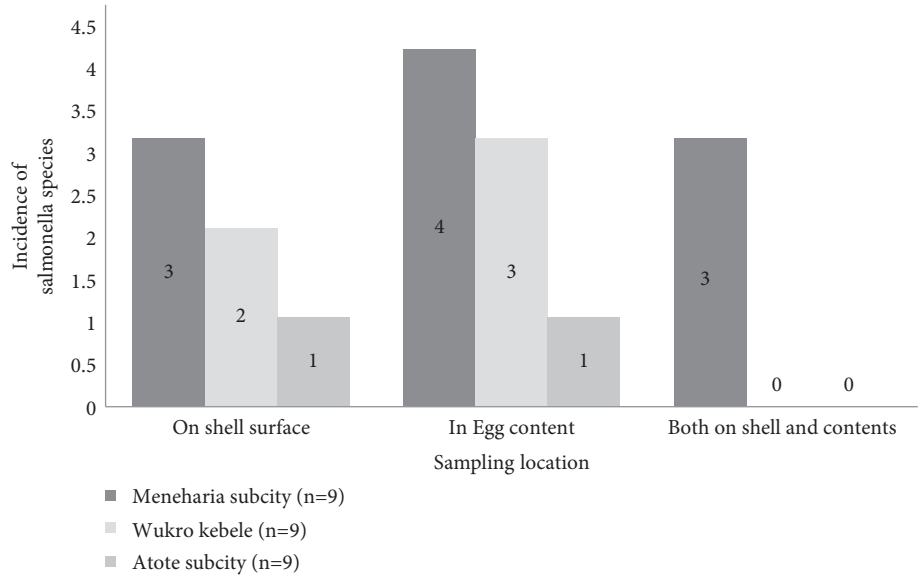

Figure 3: Incidence of Salmonella species in shell surface rinsates and contents of egg samples collected from markets in Hawassa.

incidence of salmonellae on 25 (6.3%) and 27 (6.8%) of the shell and contents of the egg samples, respectively. Therefore, the incidence of salmonellae in the shell rinsate of the egg samples in this study was slightly lower than that of samples in the report, but the incidence in the egg contents was higher. In a study of 16 egg samples from poultry farm and retail market, Chaemsanit et al. [28] reported the isolation of salmonellae from the shells of two egg samples (12.5%), but not from the egg contents, which is higher than the incidence on the eggshell of samples in this study. Likewise, Parveen et al. [64]also reported the finding of isolates related to *Salmonella* species from the shell surface rinsates of four (11.11%) and the yolk of one of 36 (2.8%) samples. Therefore, the incidence of salmonellae in the shell rinsates and contents of the egg samples from the report were higher than those of samples in this study.

The natural habitat of non-typhoidal salmonellae is the intestinal tract of various animals including poultry. Following excretion in feces, they may end up in the environment where cross-contamination into food chain will lead to return back to the intestine of consumers, and the cycle continues [14]. The serovar most commonly associated with the consumption of undercooked egg is S. enteritidis and *S.* typhimurium [65]. The inherent oviducal contamination of eggs from an infected laying hen by these serovars before being laid is a widely accepted phenomenon [10, 66]. According to USDA [3],the detection of salmonellae on food item is considered as potentially hazardous and unacceptable.

4. Conclusion

The mean MABC of the egg samples from all locations was higher than 9 $\log_{10}$ CFU/ml on both shell surfaces and internal contents, suggesting incipient spoilage and gross defects in good agricultural practices and hygienic handling in the farm and retail market and prolonged storage at favorable extrinsic parameters for microbial growth. The overall mean SC of the contents of the egg samples was also higher than 7 $\log_{10}$ CFU/ml, a value in the potentially hazardous. The overall mean YMC of the contents of the egg samples (5.52 $\log_{10}$ CFU/ml) was consistent with those of other microbial load parameters indicating poor hygienic handling and prolonged storage time. No fungal growth was detected in the contents of all of the egg samples from HUPF

indicating the relatively shorter duration of storage time as compared with those from other sampling locations. The dominant mesophilic aerobic bacterial genera of both shell and internal contents of the egg samples were dominated by *Pseudomonas*, *Micrococcus,* and *Staphylococcus.* Isolates related to Shigellae were found in the contents of two egg samples from retail markets (one each from two locations) raising safety concerns. Likewise, isolates related to *Salmonella* species were detected in the eggshell rinsate and contents of 6 (10%) and 8 (13.8%) of the egg samples. To minimize the level of contamination of eggs by spoilage and pathogenic microorganism, prevention and control measures should be in place starting from the production farm and maintained through the market to the consumer level.

Abbreviations

AMBC: Aerobic mesophilic bacterial count
EMB: Sine methylene blue agar
EC: Enterobacteriaceae count
FCC: Fecal coliform count
IMVC: Indole, methyl red, Voges Proskauer, and citrate
PDA: Potato dextrose agar
SC: Staphylococcal count
SIM: Sulfide indole motility
TCC: Total coliform count
VRBA: Violet red blue agar
YMC: Yeast and mold count.

Data Availability

All supporting data for this work are included in the main manuscript. The original data generated and/ or analyzed during this work can be obtained from the corresponding author on reasonable request.

Consent

Not applicable.

Conflicts of Interest

The authors have conflicts of interest to declare.

Authors' Contributions

AD participated in the drafting of the work plan and did all the laboratory work; AM designed the work, supervised the laboratory work, and wrote the manuscript; MA participated in the data analysis and interpretation; and BD participated in the final edition of the manuscript. All authors read and approved the final manuscript.

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Supplementary Materials

The results of the statistical analysis based on the original dataset are included as supplementary tables 1 to 6 enclosed as zip file. Supplementary tables 1 to 3 are for the microbial load parameters of the eggshell surface rinsates, while supplementary tables 4 to 6 are for the microbial load parameters of the egg contents. (*Supplementary Materials*)

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