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FULL PAPER

High levels of bacterial and antimicrobial drug residues contamination in chicken eggs for human consumption in Morogoro municipality, Tanzania

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Abstract

Eggs represent one of the most important low-cost sources of high-quality animal protein and minerals. However, the occurrence of contamination with antimicrobial drug residues and antimicrobial-resistant microorganisms has been increasing and represents a serious food safety risk. This study was carried out to assess the microbial and antimicrobial drug residue contamination of chicken eggs commercialized in selected neighbourhoods in Morogoro municipality, Tanzania. A total of two hundred and fifty-five eggs were sourced from forty-four stores in ten neighbourhoods and were examined for bacterial and antimicrobial drug residue contamination using standard microbiological assays, and the *Bacillus subtilis* ATCC 3491 as the test organism. Bacterial contamination was detected in 53.3% (136/255) of the egg yolk samples, with significant differences between sampling sites (p = 0.001). Bacterial total viable counts in the contaminated samples ranged from 10^2 to 10^9 CFU/mL, with a mean value of 6.42×10^8 CFU/mL (CI 95% = 1.45×10^8 – 1.60×10^8 CFU/mL). The most prevalent bacteria were *Bacillus* spp. (39.7%), followed by the Coagulase-negative *Staphylococcus* (16.9%), *Escherichia coli* (8.8%), *Streptococcus* spp. (5.9%), *Staphylococcus aureus* (4.4%), *Enterobacter* spp. (3.7%), *Salmonella* spp. (3.0%) and *Pseudomonas* spp. (1.5%). Antimicrobial drug residues were detected in 40.8% (104/255) of the samples in egg white and nil (0%) in the yolk. The contamination with antimicrobial residues showed significant differences between sample collection sites (p = 0.000), but not with bacterial counts (p = 0.862) and isolated bacteria in each sample collection site (p = 0.497). The presence of various bacteria and drug residues above permissible maximum residue levels in eggs predisposes consumers to poison and antimicrobial-resistant bacteria.

1. Introduction

Poultry eggs represent an important low-cost source of animal protein and other nutrients including vitamins, zinc, phosphorus and cholesterol for most African populations (FAO, 2008). In Tanzania, poultry farmers keep chickens either for the eggs, the meat, or both (Nonga *et al*., 2010). Microorganisms, especially bacteria, make use of the highly nutritious content of eggs as they represent ideal substrates for microbial proliferation. Microbial contamination of poultry eggs significantly impacts the industry by way of economic loss and can also result in infections and illnesses (Okorie-Kanu *et al*., 2016; El-Kholy *et al*., 2020).

Among the major bacteria associated with foodborne diseases, *Salmonella enterica* serovar Enteritidis is the most commonly implicated in poultry egg-related illnesses (Saha *et al*., 2012; Mahdavi *et al*., 2012). Other bacteria associated with the contamination of chicken and their eggs include *Listeria monocytogenes*, *Campylobacter jejuni* and *E. coli* (USDA-FSIS, 2015). These bacteria are known to contaminate both the eggshell and the egg content, which may occur before oviposition when the eggshell is not fully formed and the reproductive organs such as the ovary and posterior oviduct are infected (Svobodová and Tumová, 2014), or can result from contact of the eggshell with the faecal-contaminated environment during oviposition (Al-Bahry *et al*., 2012). Egg contamination can also occur after oviposition by contact of the egg with unsuitable environmental conditions including poor hygiene during handling and storage, as well as production and

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marketing sites (Lacerda *et al*., 2013; Salihu *et al*., 2015).

The occurrence of bacterial diseases in poultry, both in industrial and in small scale systems, prompts producers to use antimicrobial agents either as prophylactic, therapeutic or to improve feed conversion efficiency (growth promotion or increasing yield performance) (Nonga *et al*., 2010; Alaboudi *et al*., 2013). However, the injudicious use of antimicrobial drugs, which is rather common in the African continent (Darwish *et al*., 2013; FAO, 2014; Mensah *et al*., 2014), can lead to the occurrence of drug residues, particularly in eggs (Goetting *et al*., 2011). The quantity of antimicrobial residues deposited between the egg yolk and/or albumen depends on the physicochemical properties of the antimicrobial agent including its plasma proteins binding potential, its hydrophobicity or hydrophilicity and the physiology of egg formation (Giorgi *et al*., 2000; Kan and Petz, 2000).

Differently from most developed countries, the issue of antimicrobial residues in foods of animal origin has rarely been a serious concern in Africa where access to food, independent of its quality, appears to be a more pressing issue. It is estimated that the prevalence of veterinary drug residues in foods of animal origin in some African countries can be as high as 94%, compared to less than 1% in European countries (Darwish *et al*., 2013; Mensah *et al*., 2014). Humans can develop allergies, cancer, changes in the intestinal microbiota and antimicrobial resistance as a result of exposure to antimicrobials through contaminated food (Donoghue, 2003; Phillips *et al*., 2004).

The growing population and the resulting increased demand for food have led to the need for increasing poultry and egg production in Tanzania (FAO, 2008; Mubito, Shahada, Kimanya *et al*., 2014). Residents of Morogoro municipality usually purchase eggs from local markets and resellers (Nonga *et al*., 2010), where packaging and storage practices are often inadequate, heartening contamination and multiplication of microorganisms (Fardows *et al*., 2016; Rocha *et al*., 2016). Additionally, egg consumption practices by local populations vary from preparing with or without heat treatment for short or longer periods of time, to simply row consumption, depending on personal preferences or medical advice.

This study was developed to assess the bacterial contamination and the occurrence of antimicrobial drug residues in raw chicken eggs commercialized for human consumption in Morogoro municipality, Tanzania. It is expected that the study will inform the local population and decision-makers on the prevailing situation, which will allow the adoption of informed appropriate risk containment measures for general public health protection.

2. Materials and methods

2.1 Study location and eggs samples collection

The Morogoro municipality is located 200 km west of the city of Dar-Es-Salaam. The population of Morogoro is estimated at over 315,800 (Ernest *et al*., 2017). The climatic conditions are characterized by hot weather and frequent rainfall, with minimum and maximum temperatures averaging 16°C and 33°C, respectively (Ernest *et al.,* 2017). Egg samples were sourced from forty-four (44) reseller shops in ten (10) neighbourhoods of Morogoro municipality (Figure 1 and Table 1). A total of 255 eggs were purchased during morning hrs, between May and June 2017. Each egg was maintained in a separate sterile plastic zip lock bag and transported on ice in a thermal box to the Microbiology Laboratory of the College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, and processed within 2 hrs upon purchase.

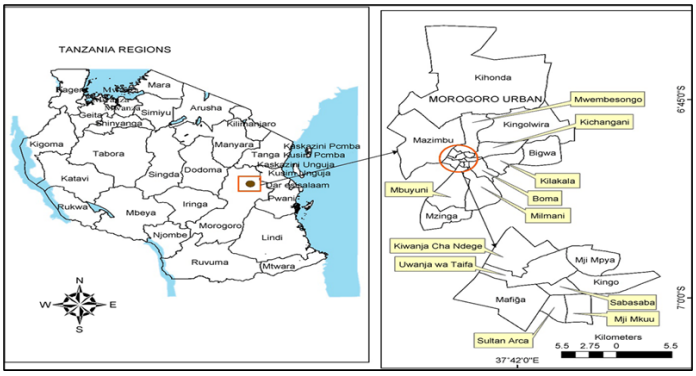

Figure 1. Geographical location of sample eggs collection sites in Morogoro municipality, Tanzania. Source: Ernest *et al*. (2017).

Table 1. Sampling sites and number of sample eggs sourced

Neighbourhoods	Number of sample collection sites	Total eggs sourced
Biguwa	5	25
Boma	3	30
Kichamgane	5	22
Kihonda	5	20
Kila Kala	5	24
Magado	3	29
Mazimbo	3	30
Misufine	5	25
Morogoro town	5	25
Saba Saba	5	25
Total	44	255

2.2 Microbial contamination testing

2.2.1 Sample preparation

Sample eggs were soaked with 70% (v/v) ethanol for

5 mins and then thoroughly cleaned with a sterile cotton hand towel. Each egg was then gently cracked using sterile forceps. Egg white was carefully and thoroughly separated from the yolk and each portion was placed into a sterile falcon tube.

For bacterial assessment, serial dilutions (10^{-1} to 10^{-9}) were prepared following standard bacteriological procedures. According to Spitzer (2016) and Lacerda *et al*. (2013), egg white contains substances with antibacterial activity, including lysozyme, which prevents the multiplication of bacteria, and other factors, such as the albumin viscosity and the alkaline pH, that hinder the survival of various microorganisms. Therefore, a bacterial contamination assessment was performed only on the egg yolk. For such, 10 mL of each egg yolk sample was mixed with 90 mL of sterile 0.1% peptone water, resulting in a 10^{-1} concentration solution. The following 10^{-2} to 10^{-9} concentration solutions were prepared by transferring 1 mL of the freshly prepared solution to the next tube containing 9 mL of sterile 0.1% peptone water. All tubes were gently shaken to obtain homogenous mixtures before transferring to the next tube.

For the isolation and identification of *Salmonella*, egg samples were enriched in Selenite-Cysteine medium and incubated for 24 hrs at 37°C before inoculation in the MacConkey agar (MaC) medium. A brief modification was made to the standard protocol of ICMSF (1986) for the detection of *Salmonella*. In the pre -heating stage, instead of 25 mL of sample in 225 mL dilution (buffered water), 10 mL in 90 mL was used since sample egg yolks were only 15 - 17 mL maximum.

2.2.2 Bacterial isolation and identification

The bacterial isolation and identification were carried out as shown in Figure 2. Purification and colony morphology examination (shape, size, colour and outlining) steps were performed according to Breed *et al*. (1957) and Spencer and Spencer (2001). In summary, 0.1 mL of the 10^{-1} sample dilution mixtures were streaked on three different culture media: Nutrient Agar (NA) for a wide range of non-fastidious bacteria; MaC for Enterobacteriaceae, and Brain Hearth Infusion agar (BHI) for fastidious and as well non-fastidious bacteria (Thermo Fisher, Oxoid Ltd, Basingstoke, UK). All plates were incubated in an inverted position in a bacteriological greenhouse (Shimadzu, Tokyo, Japan) at 37°C for 24 to 48 hrs. Subsequently, the grown bacterial colonies were subcultured onto Muller Hinton agar (MH). For colonies purification, Blood agar base (BA) for the isolation of haemolytic and non-haemolytic bacteria, Xylose Lysine Deoxycholate agar (XLD) and Salmonella-Shigella agar (SS) for the isolation and differentiation of the Enterobacteriaceae group were used. All media were from Thermo Fisher, Oxoid Ltd, Basingstoke, UK. All the isolated bacteria were further identified or confirmed through Gram staining and biochemical tests.

2.2.3 Determination of bacterial viability

For total viable counts (TVC), 1 mL of each 10^{-1} to 10^{-9} culture dilution was pipetted and spread on sterile Petri dishes containing NA culture medium (Thermo Fisher, Oxoid Ltd, Basingstoke, UK), and incubated in a bacteriological greenhouse (Shimadzu, Tokyo, Japan) at 37°C for 24 hrs. Culture plates showing growth of colonies between 30-300 CFU/mL were selected and screened using an electronic colony counter (Stuart SC6, Staffordshire, UK). The TVC was grouped into categories according to Mahdavi *et al.* (2012) and USDA -FSIS (2013).

2.2.4 Bacterial confirmation tests

2.2.4.1 Gram test

For bacterial confirmation, two to three

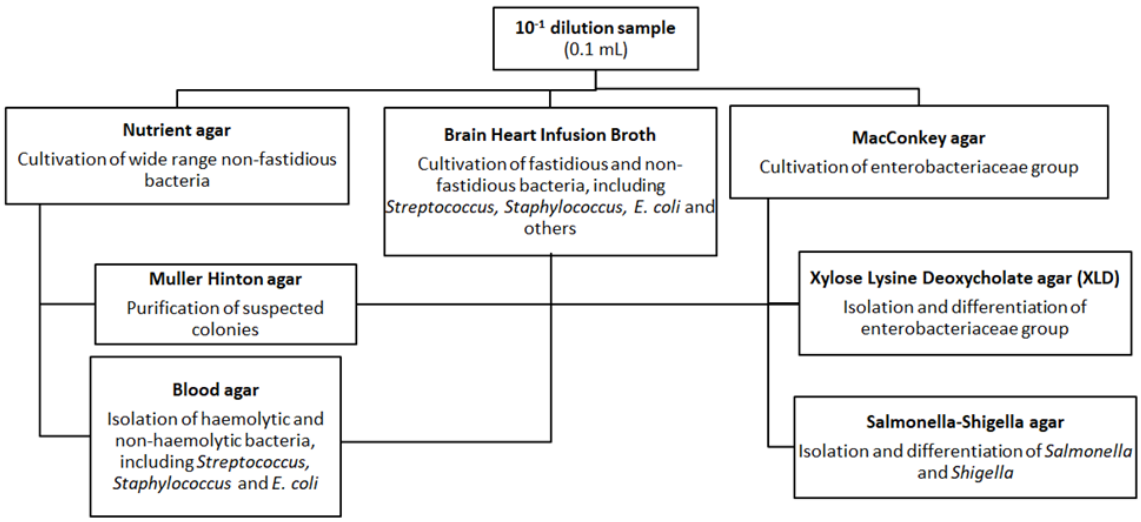

Figure 2. Schematic representation of the bacterial isolation and identification procedure

morphologically suspected colonies were aseptically collected from culture plates and placed on a glass slide, followed by the addition of 2 to 3 drops of saline and thorough mixing. The smears were then fixated on the flame on a Bunsen burner, stained with Gram stain (Thermo Fisher, Oxoid Ltd, Basingstoke, UK) and examined under 1000× magnification on a light microscope.

2.2.4.2 Coagulase and catalase test

The coagulase and catalase tests were performed to confirm and differentiate the presence of Gram-positive bacteria, particularly *Staphylococcus* and *Streptococcus*.

Typical and atypical colonies were transferred from culture plates using a sterilized loop to glass slides containing approximately 0.3 mL of sterile rabbit plasma. After thorough mixing, an examination was performed for clot formation. Bacteria that promoted serum coagulation were considered coagulase-positive.

For the catalase test, hydrogen peroxide solution was applied to the bacterial colonies on a glass slide with the aid of sterile pipette tips. The formation of air bubbles demonstrated characteristic catalase-positive microorganisms.

2.2.4.3 Biochemical tests

The biochemical confirmation tests Voges-Proskauer, Indole, Citrate, Urea, Triple Sugar Iron (TSI) and Lysine Decarboxylase (LDC) test, were performed for confirmation of Gram-negative bacteria. For this purpose, two to three colonies previously grown on MaC, SS, BA and XLD were collected aseptically and inoculated by drilling and streaking into test tubes containing the appropriate media. Colonies that were retrieved from the MaC medium were additionally incubated in 9 mL of MaC broth for 24 hrs at 37°C to perform the indole test using Kovacs reagent. In this test, the formation of a red ring on the surface of the medium was indicative of a positive indole test, which confirmed the presence of *E. coli*. Confirmation of TSI results was through the detection of different fermenting agents of glucose, lactose and/or sucrose, gas production and hydrogen sulphide formation. The urea test was considered positive when the medium changed from yellow to red/pink, with or without gas production within 24 hrs at 37°C. A change of colour from yellow to purple was considered positive for the LDC test after 48 hrs of incubation (Talaiekhozani *et al*., 2015).

2.3 Antimicrobial drugs residues testing

For the detection of antimicrobial drug residues in egg white and yolk, a bacterial growth inhibition test method using *Bacillus subtilis* ATCC 3491 as a test organism was performed on petri dishes with MH medium.

A suspension of the test organism at a concentration approximately equal to McFarland standard turbidity (equivalent to 3×10^8 cells/mL) (Kabir *et al*., 2004) was inoculated into the culture media using sterile cotton swab sticks. The uniformly, thoroughly swabbed culture media was then allowed to dry for up to 5 mins before wells were cut with a cork borer, and approximately 50 µL of egg yolk or egg white were pipetted into the wells and the plates were incubated at 37°C for 24 hrs. After incubation, the plates were evaluated for the presence or absence of clear growth inhibition zones around the wells.

Oxytetracycline solution (10%) from Farmers Africa, Zambia, and normal saline buffer were used as positive and negative controls respectively. Controls were included on each plate and, positive inhibition of bacterial growth was considered for wells with clear growth inhibition on a diameter equal to or greater than 1 mm, measured under reflected light using a graduated ruler. The plates were examined to detect the presence or absence of growth inhibition zones around the samples.

2.4 Data analysis

Data were analysed using the IBM Statistical Package for Social Sciences (SPSS) software version 20.0 (Chicago, SPSS, Inc. 2011). A Cross-frequency table and descriptive analysis (means and standard deviations) were performed. A Chi-square test was used to analyse the variations in bacterial and antimicrobial contaminations between the sample collection sites. The differences in antimicrobial inhibition values between the categorical variables were analysed using the Kruskal -Wallis One-Way test. The level of significance for all the analyses was determined at an alpha value of 0.05.

3. Results

3.1 Microbial contamination

More than half of the analysed egg yolk samples, 53.3% (136/255), showed bacterial contamination. As shown in Table 2, bacterial TVC of contaminated samples ranged from 10^2 to 10^9 CFU/mL, with mean value of 6.42×10^8 CFU/mL (CI 95% = 1.45×10^8 – 1.60×10^8 CFU/mL). Significant differences in bacterial contamination ($p = 0.001$) and the distribution of bacterial count category ($p = 0.000$) were observed among sample collection sites and neighbourhoods.

As shown in Table 3, Boma and Mazimbu had the highest (7.5%) microbial contamination rate, while the

Table 2. Bacterial total viable counts of tested egg yolks

Bacterial count category (CFU/mL)	Frequency	Count levels (CFU/mL)		
	n (%)	Mean	Min. – Max.	CI95% lower – Upper
< 10 – 30	119 (46.7)	N/A	N/A	N/A
30 – 1×10^2	ND	N/A	N/A	N/A
1.01×10^2 – 1×10^3	10 (7.4)	4.52×10^2	1.40×10^2 – 8.70×10^2	2.61×10^2 – 6.42×10^2
1.01×10^3 – 1×10^4	19 (14.0)	3.37×10^3	1.02×10^3 – 9.60×10^3	2.34×10^3 – 4.40×10^3
1.01×10^4 – 1×10^5	14 (10.3)	2.85×10^4	1.03×10^4 – 7.00×10^4	1.70×10^4 – 4.40×10^4
1.01×10^5 – 1×10^6	2 (1.5)	2.32×10^5	1.90×10^5 – 2.73×10^5	2.96×10^5 – 7.59×10^5
1.01×10^6 – 1×10^7	17 (12.5)	4.65×10^6	1.05×10^6 – 9.40×10^6	3.21×10^6 – 6.08×10^6
1.01×10^7 – 1×10^8	17 (12.5)	2.56×10^7	1.38×10^7 – 4.48×10^7	2.07×10^7 – 3.06×10^7
> 1×10^8	57 (41.9)	1.52×10^8	3.00×10^8 – 2.00×10^9	1.45×10^8 – 1.60×10^8
Total	136 (53.3)	6.42×10^8	1.40×10^2 – 2.00×10^9	1.45×10^8 – 1.60×10^8

n: frequency of analysed samples, ND: Not detected, CI95%: Confidence Interval of 95%, N/A: Not Applicable.

Table 3. Frequency of bacterial contamination and categories of bacterial counts in the yolk of eggs collected from different neighbourhoods.

Neighbourhood	Positive samples	Frequency *n* (%) of Bacterial counts (CFU/mL) per category						
	n/N (%)	1.01×10^2 – 1×10^3	1.01×10^3 – 1×10^4	1.01×10^4 – 1×10^5	1.01×10^5 – 1×10^6	1.01×10^6 – 1×10^7	1.01×10^7 – 1×10^8	> 1×10^8
Biguwa	9/25 (3.5)	0 (0)	1 (0.4)	1 (0.4)	0 (0)	2 (0.8)	4 (1.6)	1 (0.4)
Boma	19/30 (7.5)	0 (0)	1 (0.4)	0 (0)	0 (0)	2 (0.8)	7 (2.7)	9 (3.5)
Kichamgane	14/22 (5.5)	0 (0)	0 (0)	0 (0)	1 (0.4)	6 (2.4)	5 (2.0)	2 (0.8)
Kihonda	1/20 (0.4)	0 (0)	1 (0.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Kila Kala	10/24 (3.9)	0 (0)	1 (0.4)	3 (1.2)	1 (0.4)	5 (2.0)	0 (0)	0 (0)
Magado	17/29 (6.7)	4 (1.6)	3 (1.2)	1 (0.4)	0 (0)	0 (0)	0 (0)	9 (3.5)
Mazimbo	19/30 (7.5)	2 (0.8)	5 (2.0	2 (0.8)	0 (0)	0 (0)	0 (0)	10 (3.9)
Misufine	16/25 (6.3)	2 (0.8)	4 (1.6)	1 (0.4)	0 (0)	0 (0)	0 (0)	9 (3.5)
Morogoro Town	16/25 (6.3)	0 (0)	2 (0.8)	6 (2.4)	0 (0)	2 (0.8)	0 (0)	6 (2.4)
Saba Saba	15/25 (5.9)	2 (0.8)	1 (0.4)	0 (0)	0 (0)	0 (0)	1 (0.4)	11 (4.3)

n/N: Number of positive samples/Total of analysed samples.

lowest contamination rate was recorded for Kihonda (0.4%) neighbourhood. All sampled eggs, except those obtained from Kihonda and Kilakala, showed bacterial contamination above 1×10^8 CFU/mL. The higher rates of such levels of contamination were observed in eggs from Saba Saba (4.3%), Mazimbo (3.9%), Boma (3.5%), Magado (3.5%) and Misufine (3.5%).

Gram-positive bacteria were the most prevalent contaminant microorganisms of the eggs tested. *Bacillus* spp. (39.7%), followed by the Coagulase-negative *Staphylococcus* (16.9%) and *E. coli* (8.8%) were the most predominant microorganisms. *Streptococcus* spp. (5.9%), *S. aureus* (4.4%), *Enterobacter* spp. (3.7%), *Salmonella* spp. (3.0%) and *Pseudomonas* spp. (1.5%) were also isolated. A total of 16.2% of the samples showed contamination with microorganisms that could not be identified through the performed tests.

Bacillus spp. showed significant occurrence differences (p = 0.000) between neighbourhoods. Samples from Boma (8.8%) followed by Mazimbo (5.9%) exhibited the highest occurrence of this microorganism, while samples from Kihonda (0.7%) showed the lowest occurrence of the bacterium (Table 4).

3.2 Antimicrobial drugs residues

The 255 analysed eggs revealed that 40.8% (104/255) had detectable levels of antimicrobial activity in the albumen and nil (0%) in the yolk (Table 5). The proportion of antimicrobial activity showed significant ($p<0.001$) differences between the sample collection sites. Samples from Boma (80.0%), Misufine (64.0%) and Mazimbo (56.7%) revealed the highest rate of antimicrobial activity while the lowest rate was observed in samples from Biguwa and Morogoro Town, both with 8.0%.

The diameter of the bacterial growth inhibition zone for all the samples varied between 8 and 16 mm, with an average value of 11.26±1.68 mm. The largest diameters of bacterial growth inhibition were observed for the egg samples from Biguwa (12.00 mm), Kihonda (10.13 – 14.20 mm), Mazimbo (10.07 – 12.04 mm) and Misufine

Table 4. Prevalence of bacterial contamination of egg yolks from different neighbourhoods of Morogoro municipality

Neighbourhood	Isolated Microorganisms, n (%)								
	Staphylococcus spp.	*S. aureus*	*Streptococcus* spp.	*Escherichia coli*	*Enterobacter* spp.	*Bacillus spp.*	*Salmonella* spp.	*Pseudomonas* spp.	Others [a]
Biguwa	2 (1.5)	0 (0.0)	0 (0.0)	3 (2.2)	0 (0.0)	4 (3.0)	1 (0.7)	0 (0.0)	6 (4.4)
Boma	4 (3.0)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	12 (8.8)	0 (0.0)	0 (0.0)	1 (0.7)
Kichamgane	6 (4.4)	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)	6 (4.4)	2 (1.5)	2 (1.5)	0 (0.0)
Kihonda	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	0 (0.0)	0 (0.0)
Kila Kala	1 (0.7)	1 (0.7)	0 (0.0)	3 (2.2)	1 (0.7)	6 (4.4)	0 (0.0)	0 (0.0)	0 (0.0)
Magado	0 (0.0)	1 (0.7)	2 (1.5)	2 (1.5)	1 (0.7)	3 (2.2)	0 (0.0)	0 (0.0)	0 (0.0)
Mazimbo	1 (0.7)	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)	8 (5.9)	1 (0.7)	0 (0.0)	3 (2.2)
Misufine	1 (0.7)	1 (0.7)	0 (0.0)	1 (0.7)	2 (1.5)	5 (3.7)	0 (0.0)	0 (0.0)	6 (4.4)
Morogoro Town	8 (5.9)	2 (1.5)	3 (2.2)	1 (0.7)	1 (0.7)	3 (2.2)	0 (0.0)	0 (0.0)	3 (2.2)
Saba Saba	0 (0.0)	0 (0.0)	3 (2.2)	0 (0.0)	0 (0.0)	6 (4.4)	0 (0.0)	0 (0.0)	3 (2.2)
Total	23 (16.9)	6 (4.4)	8 (5.9)	12 (8.8)	5 (3.7)	54 (39.7)	4 (3.0)	2 (1.5)	22 (16.2)

[a]Bacteria that could not be identified by the performed methods/procedures

Table 5. Residues of antimicrobial substances detected in albumen content

Local	Positive samples*	Growth inhibition zone** (mm)		
	n/N (%)	Mean±SD	Min. – Max.	CI95% lower – Upper
Biguwa	2/25 (8.0)	12.00±0.00	12.00	12.00
Boma	24/30 (80.0)	11.71±0.99	11.00 – 13.00	11.29 – 12.13
Kichamgane	9/22 (40.9)	10.56±0.88	9.00 – 12.00	9.87 – 11.23
Kihonda	6/20 (30.0)	12.20±1.94	10.00 – 15.00	10.13 – 14.20
Kila Kala	10/24 (41.7)	10.40±1.17	9.00 – 13.00	9.56 – 11.24
Magado	6/29 (20.7)	10.50±1.52	8.00 – 12.00	8.91 – 12.09
Mazimbo	17/30 (56.7)	11.06±1.92	8.00 – 14.00	10.07 – 12.04
Misufine	16/25 (64.0)	12.69±1.96	8.00 – 16.00	11.64 – 13.73
Morogoro town	2/25 (8.0)	8.50±0.71	8.00 – 9.00	8.50
Saba Saba	12/25 (48.0)	10.25±1.14	8.00 – 12.00	9.52 – 10.97
Total	104 (40.8)	11.26±1.68	8.00 – 16.00	11.29 – 12.13

SD: Standard deviation; *n*/N: number of positive samples/total of analysed samples, CI95%: Confidence Interval of 95%. *Values differ statistically between the locals by the Chi-square test (P = 0.00), **Values differ statistically between the locals by Kruskal-Wallis One-Way nonparametric test (P = 0.00).

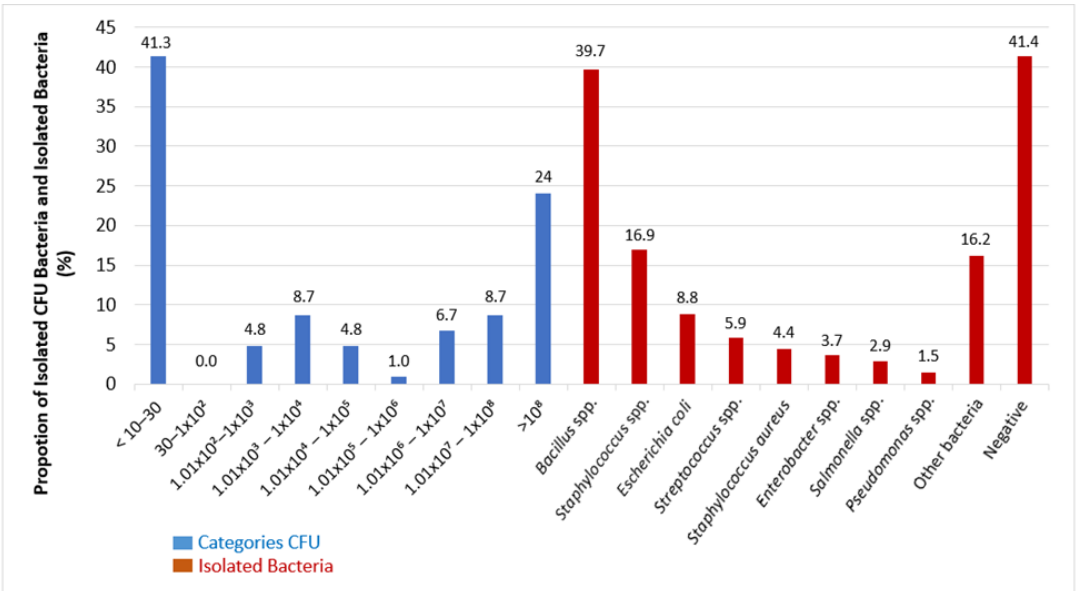

Figure 3. Proportion of CFU bacterial categories in drug residues positive samples and proportion of isolated egg contaminant bacteria.

(11.64 – 13.73 mm), while the smallest growth inhibition zones were observed for egg samples from Morogoro town (8.50 mm).

Of the 40.8% positive samples for antimicrobial drug residues, most were samples with bacterial count categories of > 1×10^8 CFU/mL and 1.01×10^5 to 1×10^6 CFU/mL.

Bacillus spp. (23.1%) and coagulase-negative *Staphylococcus* (8.7%) were the predominant isolated bacteria (Figure 3). No significant difference was detected in the occurrence of antimicrobial residues between bacterial count categories ($p = 0.862$) and isolated bacteria ($p = 0.497$).

4. Discussion

4.1 Microbiological contamination

Freshly laid eggs are generally sterile. However, several studies have indicated that microorganisms can be found on the eggshell and content (Mayes and Takeballi, 1983; USDA-FSIS, 2013; Tizo *et al*., 2015). In this study, high levels of bacterial contamination on egg yolks were found, along with significant differences in total bacteria viable counts ($p\leq0.05$) between samples. Of all the bacteria-contaminated egg samples, 33.2% showed allowable levels of contamination, while 66.9% of the samples were unsatisfactory, according to international standards (New Zealand Food Safety Authority, 1995). These results may reflect poor local storage and selling practices, as the eggs were stored and retailed on open cardboard containers; exposed to open air at room temperature; subject to dust, insects, intense heat and direct sunlight. In addition, most farmers in Morogoro municipality use traditional production systems, where the possibility of contamination of eggs with undesirable agents is high due to frequent handling and no use of sophisticated equipment (FAO, 2008; Mubito, Francis, Martin *et al*., 2014; Nonga *et al*., 2010). According to Chaemsanit *et al*. (2015), poor storage and selling practices may aggravate the level of bacterial contamination, and lead to the rupture of the physical barriers of the eggs. The recommended international code of good hygiene practices for eggs and egg products (CAC/RCP 15 – 1976) (CAC, 2007), states that eggs must be collected, handled, stored and transported in a manner that minimizes microorganism contamination and/or damage to the egg or eggshell, with appropriate attention to time-temperature fluctuations, which are among the key factor for the growth of existing microorganisms.

Most of the isolated bacteria in this study are pathogenic to humans and they can persist on and in the egg for a longer period under harsh conditions. Coagulase-negative *Staphylococcus* and *E. coli* were the most prevalent out of the isolated Gram-positive and Gram-negative bacteria respectively. These findings can be attributed to human poor handling and hygiene (Mahdavi *et al*., 2012), and faecal contamination of the egg-laying environment (Eman and Saad, 2015). It is also known that the sudden change in temperature to which the egg is subject after laying, creates a negative pressure inside the egg content and promotes its contraction. This contraction pulls inside the egg-content, bacteria present in the environment or on the egg surface through the shell (Befungi *et al.,* 1999). Likewise, high bacterial load in the eggshell, associated with egg cleaning and washing right after it is laid can considerably contribute to a quick passage of bacteria into the egg content, since the cleaning procedures facilitate the removal of the protective egg cuticle (Abdullah, 2010; Leleu *et al*., 2011; Al-Bahry *et al*., 2012).

The recorded high contaminations of eggs with Gram-positive bacteria are consistent with the fact that Gram-positive bacteria can tolerate unfavourable conditions such as low moisture content (Chaemsanit *et al.*, 2015), compared to Gram-negative bacteria which are less impervious to the antimicrobial defences of egg white (Fardows *et al*., 2016), and more sensitive to the low pH ($pH \leq 6.2$) of the yolk (Cader *et al*., 2014).

The occurrence of bacteria such as *Salmonella*, *E. coli* and *Staphylococcus* in yolk shows that consumers, particularly children and immunologically impaired individuals are at risk of developing foodborne disorders including diarrhoea, urinary tract infections, and other severe illnesses (Ghasemian *et al*., 2011; El-Kholy *et al*., 2020) as a result of consuming the eggs, particularly, if they are consumed uncooked or undercooked. Additionally, the presence and multiplication of non-pathogenic microorganisms in the eggs promote the degradation of their quality, reduction of shelf life and reduction of their nutritional value (ICMSF; 1986; Abdullah, 2010).

In Tanzania, data on outbreaks of food poisoning are scarce, possibly due to underreporting. However, the results of the present study, as well as other studies conducted in other African countries (Cader *et al.,* 2014; Eman and Saad, 2015; Salihu *et al*., 2015; Okorie-Kanu *et al*., 2016; Jambalang *et al*., 2017), suggest that contamination of eggs by different microorganisms is common. These findings are probably related to traditional production systems, hot temperatures, lack of adequate good practices in poultry production, and lack of systems for monitoring farms and markets or retail activities. Therefore, the results of this study highlight

the need for improving the hygiene conditions from production to distribution and retail of eggs in order to reduce the bacterial load on commercial eggs for human consumption, increase their shelf life, and reduce risks to public health. Consumption of raw or undercooked eggs in the study area should be discouraged.

4.2 Antimicrobial drugs contamination

Widespread and indiscriminate use of antibiotics for the treatment of bacterial diseases or as feed additives for domestic animals and birds is common in many African countries, resulting in one of the biggest challenges to public health (Darwish *et al*., 2013; Mensah *et al*., 2014). In this study, antimicrobial substances were detected only in egg whites, with 40.8% of positivity. Even though there were significant differences in the presence of antimicrobial drug residues between the sampling locations ($p \leq 0.05$), differences were however not statistically significant between the bacterial count categories and the isolated bacteria ($p \geq 0.05$). Out of the antimicrobial drug residues positive samples, most (80.0%) were from the Boma neighbourhood. The result suggests an association between locally occurring diseases commonly treated with specific antibiotics, and egg importation from other municipalities (Mubito, Francis, Martin *et al*., 2014).

Our finding of the non-occurrence of antimicrobial residues in the yolk may be related to factors that include pharmacokinetics characteristics of the commonly used drugs, and layers-related factors, as described by Kan and Petz (2000), Kan (2003) and Goettin *et al.* (2011). The antimicrobial contamination of eggs white found in this study was above those reported in previous studies conducted by Nonga *et al*. (2010) in the same municipality. In their study, Nonga *et al*. (2010) examined eggs from small producers and obtained 21.4% of positivity for antimicrobial residues in egg white. Another study conducted by Mubito, Francis, Martin *et al*. (2014) in commercial eggs in Dar-Es-Salam, Tanzania obtained 100% positivity for sulfadiazine residues, with 29.2% of the positive samples above the maximum admissible levels.

Other studies in neighbouring African countries have reported contrasting results. A study by Idowu *et al*. (2010) and another one by Hakimzadegan *et al*. (2014) described antimicrobial residues in the yolk, rather than in egg white. The presence of antimicrobial residues in the yolk, even in a greater proportion than in the white, was reported by Donoghue *et al*. (1996), Donoghue and Hairston (2000), Donoghue (2003), Alm El Dein and Elhearon (2010) in experimental studies, demonstrating high levels of antimicrobial residues in egg white on the first day after administration, and gradual decrease along the withdrawal period, and gradually increase in the yolk during the same period. In general, these findings are justified by the fact that the yolk formation time is approximately 6-8 days, while most of the egg white components, particularly albumen, are formed and incorporated within 24 hrs (Giorgi *et al*., 2000; Kan, 2003).

Other influencing factors for the presence or absence of antimicrobial residues in egg contents include the screening methods. Ehsani and Hashemi (2015), Navrátilová (2008) and Shahbazi *et al*. (2015), described that although the agar well-diffusion test has the advantage of wide detection spectrum, simplicity to perform and its low cost, as well as its applicability to screening a large number of samples, it presents the limitation of being only qualitative, requires a long incubation period (2.5-72 hrs), and it does not allow the identification of specific antimicrobials. It is also well known that the presence of lysozyme in albumin can influence the positivity of antimicrobial residues found in egg white, rather than in yolk (Cháfer-Pericás *et al*., 2010; Idowu *et al*., 2010; Hakimzadegan *et al*., 2014). Therefore, according to these authors, it is recommended to screen for antimicrobial residues in the yolk instead of the white. Navrátilová *et al*. (2014) report that natural antimicrobial substances present in higher concentrations in the white than drug residues may cause false results. Other factors generally cited as influencing egg content antimicrobial activity is the sample preparation, the amount of the sample inoculated into the wells, and the type of test microorganism used. Cháfer-Pericás *et al*. (2010) refer that although *B. subtilis* is an excellent test microorganism due to its high sensitivity to β-lactam antibiotics, it presents a low sensitivity to other antimicrobial agents such as macrolides, sulfonamides, tetracyclines, and chloramphenicol.

However, despite the limitation of the Agar Diffusion Test, that was used in our study, it is a generally recommended essay for screening antimicrobial residues in animal products, highlighting its possibility to detect several classes of antibiotics, often close to the JEFCA maximum permissible limits (Ezenduka *et al*., 2014), which in turn, provides an insight into the scenario of possible antimicrobial misuse in poultry farmers in the study area. As suggested by other authors, the use of methods such as High-Performance Liquid-based Chromatography (HPLC) is recommended (Haken *et al*., 2013) to confirm and quantify the presence of antimicrobial drug residues in commercial eggs in Morogoro, Tanzania, and assure public safety.

The detection of antimicrobial drug residues in egg

white and not in the yolk suggests under dosage administration of antimicrobial agents to the laying hens even after the complete formation of the egg. This implies that good veterinary practices continue not observed in relation to drug withdrawal time, as previously reported by Nonga *et al*. (2010), Mubito, Francis, Martin *et al*. (2014) and Mubito, Shahada, Kimanya *et al*. (2014). As indicated by Beyene (2016), the inefficient monitoring and control of drug residues in food of animal origin in Tanzania, and the unusual law enforcement actions at the farm level, may constitute one of the important reasons for the documented results.

5. Conclusion

The high level of pathogenic and non-pathogenic bacterial contamination of eggs in Morogoro municipality, Tanzania is indicative of poor hygiene practices along the chicken egg chain. This can result in shortened egg shelf-life and is a potential hazard for egg consumers and public health. The presence of antimicrobial agents in the eggs from all sampling sites indicates widespread inadequate use of antimicrobial drugs in laying hens in Morogoro. Effective measures to control antimicrobial drug use in laying hens should be pursued. Larger studies with the identification and quantification of antimicrobial agents in eggs are also advocated for.

Conflict of interest

The authors declare no conflict of interest.

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