



## Prevalence and molecular characterization of non-typhoidal *Salmonella* in poultry of Dar es salaam, Tanzania

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### Keywords

Non-typhoid  
*Salmonella*;  
Poultry;  
back yard chicken;  
contamination;  
Dar es Salaam

### Abstract

Poultry is one of the common source of non-typhoidal *Salmonella* and poultry products are the major sources of human and animal infections with the *Salmonella*. A cross-sectional study was conducted to determine the prevalence of non-typhoidal *Salmonella* in live commercial and backyard chickens from retail markets and farms in Dar es Salaam city, Tanzania. The study was conducted in four districts; Ubungo, Kinondoni, Temeke and Ilala. A total of 400 chickens (120 broilers, 120 layers and 160 backyard chickens) were tested for *Salmonella*. *Salmonella* isolation and identification was carried out using bacteriological standard methods and polymerase chain reaction (PCR) targeting *Salmonella* specific *invA* gene of 118 bp. The descriptive statistics analysis was conducted using R-Software, and  $p < 0.05$  was considered statistically significant. Overall, 8% (N=32) of 400 chickens tested *Salmonella* positive. The prevalences of *Salmonella* in broilers, layers and backyard chickens were 15%, 7.5% and 3.1%, respectively. The proportions of *Salmonella* serovars detected were 78%, 16% and 3% for *S. gallinarum*, *S. typhimurium* and *S. hadar*, respectively. The findings confirmed *Salmonella* contamination in healthy chickens from markets and farms, this creates high chance for zoonotic transmission through ingestion of contaminated poultry meat. Hence call up for more researches and surveillance to monitor the risk associated with *Salmonella* infection for appropriate control measures.

### Introduction

*Salmonella* is a genus of Gram negative bacteria belonging to the family *Enterobacteriaceae* (Shimizu et al. 2014). *Salmonella* has two species which are: *Salmonella bongori* and *Salmonella enterica*

(Eng et al. 2015). The species *Salmonella enterica* has six subspecies which includes; *S. enterica*, *S. salamae*, *S. arizonae*, *S. diarizonae*, *S. houtenae*, and *S. indica* (Eng et al. 2015, Shimizu et al. 2014). *Salmonella enterica* subspecies includes over 1400

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serotypes that causes approximately 99% of *Salmonella* infections in humans and animals (Ferrari et al. 2019, Matchawe et al.2022), and are classified in two groups: typhoidal *Salmonella* and non-typhoidal *Salmonella* (NTS) (Ngogo et al. 2020)

NTS is regarded as the second largest significant foodborne pathogen globally which cause morbidity and mortality (Sharma et al. 2021). Infection of NTS in human is associated with consumption of contaminated food and is characterized by gastroenteritis and is more prevalent in Sub Saharan African countries (Ngogo et al. 2020 Siddiky et al. 2022). Poultry is the main reservoir of this pathogen, and food like milk, meat and eggs serves as a primary source which carry these pathogens and cause salmonellosis (Siddiky et al. 2022). In poultry, various NTS serovars are known to be present in the gastrointestinal tract (GIT) without showing any clinical signs increasing the chance of infection (Ferrari et al. 2019). Worldwide, NTS is estimated to cause 93.8 million cases of enteric infection and 155,000 deaths per annum and is the second major cause of diarrhea affecting mainly children under five years of age and immune-compromised hosts (Balasubramanian et al. 2018, Msemu et al. 2019, Ngogo et al. 2020, Hugh et al. 2024). However, there is limited information on the burden of NTS in commercial and backyard chickens in Tanzania with their respective serovars circulating in poultry (Munuo et al. 2022, Rukambile et al. 2021). Therefore, this study was conducted to determine the prevalence of non-typhoidal *Salmonella* in live commercial and backyard chickens from retail markets and farms in Dar es Salaam city. The findings are of interest to the respective regulatory authorities for continued surveillance and monitoring the risks associated with *Salmonella* infection for appropriate control measures.

## Materials and Methods

### Study areas

The study was conducted in Dar es salaam city (6° 46' 33.6432" S and 39° 10' 41.9736" E) involving four administrative districts namely, Ilala, Kinondoni, Temeke and

Ubungu. Dar es Salaam city is the largest commercial hub of Tanzania. Small scale (backyard henhouses) and medium to large scale poultry farming are carried out in urban and peri-urban areas, respectively. Its poultry population is estimated at 8.5 million (NBS 2021).

### Sample Size Determination

Sample size was obtained using Andrew Fishers formula (Naidoo et al. 2022).

$$n = \frac{Z^2 \times SD \times (1-SD)}{C^2}$$

where; n= sample size; Z= 1.96 at 95% confidence interval;

SD= Standard deviation 0.5; and C= Precision =5% (0.05)

$$\text{Therefore, } n = \frac{1.96^2 \times 0.5 \times (1-0.5)}{0.05^2}$$

$$n = 384.16 \sim 384$$

The *calculated* sample size was 384 but sample size of 400 was used in this study in order to increase the power for statistical analyses.

### Study Design and Sample Collection

A cross-sectional study was carried out from January 2023 to September 2023. Cloaca swabs samples from live chickens which are in food chain (broilers of 3-4 weeks old, adult layers and adult backyard chickens) were collected, where by a total of 400 chickens comprised of broilers (n=120), layers (n=120) and backyard chicken (n=160). All broilers and layers were collected from markets, while backyard chicken's cloaca swabs were collected from farms.

A list of farms and chicken markets were obtained from Agriculture, Livestock and Fisheries Division Office, from respective Local Government Authorities. The markets included Shekilango and Manzese located in Ubungu district, Kisutu and Buguruni markets in Ilala district, Stereo and Zakhem markets in Temeke district and Magomeni and Mtambani markets in Kinondoni district. A total of 30 chickens (n=15 broilers and n=15 layers) from each market were collected. For each type of chicken, 5 willing vendors were selected, and 3 chickens per each vendor prior to slaughter were randomly

sampled. Sampling was done using sterile cotton swab.

In addition, a total of 10 farms each farm with at least 100 chickens were selected from each district, and 4 chickens from each poultry farm were randomly sampled to make a total of 40 samples in each district. All swabs were aseptically collected and enclosed in a swab case containing amies transport media, in a cool box with ice pack to maintain a temperature of 4°C and transported to Central Veterinary Laboratory (CVL), department of Bacteriology for processing.

### **Salmonella Isolation and Biochemical Identification**

Isolation and identification were performed according to the Standard ISO 6579-1:2017 with slightly modifications (Ngogo et al. 2020). Briefly, a cloaca swab was dipped into 10 ml of buffered peptone water (BPW; OXOID, UK) and incubated at 37°C for 24 hours, then 0.1 ml of BPW was suspended in 10 ml of Rappaport Vassiliadis (RVB) broth (OXOID, UK) and incubated at 42°C for 24 hours. A loopful of suspension was withdrawn from RVB (OXOID, UK) and streaked on Xylose Lysine Deoxycholate agar (XLD; OXOID, UK) medium and incubated at 37°C for 24 hours. Red coloured bacterial colonies with blackish at the centre on XLD (OXOID, UK) were picked for further identification by microscopic and biochemical tests (Tripple Sugar Iron (TSI; OXOID, UK), Urease (OXOID, UK) and Citrate (OXOID, UK test). Presumptive *Salmonella* isolates were stored at -20°C in storage media containing nutrient broth (NB; OXOID) and 20% glycerol in cryovials.

### **Genomic DNA extraction**

Genomic DNA was extracted from the *Salmonella* isolates by using Zymo Research kit (USA). Briefly, *loopfuls* were withdrawn from cryovials containing stored isolates and subculture on nutrient agar (NA; OXOID, UK) at 37°C for 24 hours. Thereafter 3-5 single colonies in NA plates were picked using sterile wire loop and inoculated in 10ml tube containing NB and incubated at 37°C

for 24 hours. The rest of the procedures were performed according to manufacturer's instructions.

The quality of the eluted DNA was checked using Nanodrop spectrophotometer (Thermo Scientific™ Nano drop 2000 Waltham, USA) and then stored at -20°C for further analysis.

### **Confirmatory tests of Salmonella Isolates**

*Salmonella* isolates were confirmed by using PCR targeting a 118 bp *Salmonella* specific gene (*invA*). *S. typhimurium* ATCC 13311 (Minnesota, USA) was used as a positive control and sterile distilled water was used as negative control. PCR was performed in a final volume of 25 µl consisting of 12.5 µl of one quick 2x master mix (New England Biolabs), 0.5 µl of each primer pair (F-GCCATGGTATGGATTGTCC and R-GTCACGATAAAACCG), 8.5 µl of nuclease free water (Water for Molecular biology, BioConcept) and 3 µl (100-200 ng/µL) of DNA template. The reaction consisted of an initial denaturation at 95 °C for 6 minutes, followed by 40 cycles of 95 °C for 1 minute, 50°C for 12 seconds, and extension at 72 °C for 1 minute, with a final extension step at 72 °C for 4 minutes (Shimizu et al. 2014).

All PCR fragments were separated using 1.5% agarose gel electrophoresis stained with safe view™ classic dye (1ml, G108, abm) and electrophoresed at 100V for 40 minutes using Quick load 100 bp DNA ladder (New England, Biolabs) was used as molecular marker and visualized using UV light excitation. Image was digitalized using a Gel Documentation System (Nagar, India).

### **PCR-based Determination of NTS serovars**

Determination of NTS serovars was done using two multiplex and one singleplex PCR. The first multiplex PCR set was used to screen *Sdf* gene for *S. enteritidis*, *fliC-I* gene for *S. typhimurium*, *fliC-k* for *S. thompson* and *fliC-r* for *S. infatis* as described (Shimizu et al. 2014), (Table 1). The choice of targeted serovars was based on their importance and existence in poultry for instance *S. gallinarum* and *S. pullorum* specifically infect poultry (Xiong et al. 2018), *S. typhimurium* and *S. enteritidis* are the main serovars which cause morbidity and mortality in both

humans and animals and are widely reported worldwide (Mthembu et al. 2019, Siddiky et al. 2022). *S.thompson*, *S. hadar* and *S.infatis* have also been reported to infect both humans and animals, and cause mortality (Ye et al. 2021).

The reaction was carried out in a final volume of 25 µl composed of 5 µl multiplex PCR 5x master mix (New England, Biolabs), 0.5 µl of each primer pair (Table 1), 13 µl nuclease free water and 3 µl (100-200 ng/µL) DNA template. The reaction composed of an initial denaturation at 95 °C for 6 minutes,

followed by 40 cycles of 95 °C for 1 minute, 52 °C for 12 seconds, and extension at 72 °C for 1 minute, with a final extension step at 72 °C for 4 minutes. All PCR fragments were separated using 1.5% agarose gel electrophoresis stained with safe view™ classic dye (1ml, G108, abm) and electrophoresed at 100V for 40 minutes using Quick load 100 bp DNA ladder (New England, Biolabs) was used as a molecular marker and visualized using UV light excitation. Image was digitalized using a Gel Documentation System (Nagar, India).

**Table 1:** Multiplex PCR primers used for identification of *Salmonella enterica* serovars

| Serovar               | Genes         | Primer sequence (5'-3')                              | Amplicon size (bp) |
|-----------------------|---------------|------------------------------------------------------|--------------------|
| <i>S.Typhimurium</i>  | <i>fliC-i</i> | F-ACTCAGGCTTCCCGTAACGC<br>R-ATAGCCATTTACCAGTTCC      | 551                |
| <i>S. Enteriditis</i> | <i>Sdf</i>    | F-TGTGTTTTATCTGATGCAAGAGG<br>R-TGAACTACGTTCTTCTTCTGG | 333                |
| <i>S. Thompson</i>    | <i>fliC-k</i> | F-AACGACGGTATCTCCATTGC<br>R-CAGCCGAACTCGGTGTATTT     | 658                |
| <i>S. Infantis</i>    | <i>fliC-r</i> | F-TTGCTTCAGCAGATGCTAAG<br>R-CCACCTGCGCCAACGCT        | 413                |

The second multiplex PCR set screened for *Salmonella enterica* (*stn*) as internal control, *S. gallinarum* and *S. pullorum* (Table 2) as according to Xiong et al. (2018). The reaction was carried out in a final volume of 25 µl comprised of 5 µl multiplex PCR 5x master mix, 0.5 µl of each primer pair (Table 2), 14 µl of nuclease free water and 3 µl (100-200 ng/µL) of DNA template. The PCR amplification cycles consisted of initial denaturation at 94 °C for 5 minutes, 40 sequential cycle of 94 °C for 45 seconds, 50

°C for 45 seconds, and 72 °C for 40 seconds and a final extension step at 72 °C for 4 minutes. All PCR fragments were separated using 1.5% agarose gel electrophoresis stained with safe view™ classic dye (1ml, G108, abm) and electrophoresed at 100V for 40 minutes using Quick load 100 bp DNA ladder (New England, Biolabs) was used as a molecular marker and visualized using UV light excitation. Image was digitalized using a Gel Documentation System (Nagar, India).

**Table 2:** Multiplex PCR primers used for identification and discrimination of *S. gallinarum* and *S. pullorum*

| Primer                 | Primer sequence (5'-3')                          | Amplicon (bp) | <i>S. pullorum</i> | <i>S. gallinarum</i> |
|------------------------|--------------------------------------------------|---------------|--------------------|----------------------|
| Stn                    | F-TATTTTGCACCACAGCCAGC<br>R-CGACCGCTATATCATCACTG | 131           | Positive (+)       | Positive (+)         |
| I <sub>137-08605</sub> | F-CACTGGAGACTCTGAGGACA<br>R-GGGCAGCGAGTCTTGAGATT | 290           | Positive (+)       | Positive (+)         |
| ratA<br>ROD            | F-ATTGCTCTCGTCCTGGGTAC<br>R-TACCGATACGCCCAACTACC | 571           | Negative (-)       | Positive (+)         |

One uniplex PCR was done to screen for *S. hadar* (902 bp) (Ye et al. 2021). The PCR was carried out in a final volume of 25 µl composing of 12.5 µl one quick 2x master mix, 0.5 µl of each primer pair; *forward* (5'-GGAATAACAAAGGTGGTACT-3') and *reverse* (5'-CCTGACCTTAGAGAATGGCT-3'), 8.5 µl of nuclease free water and 3 µl (100-200 ng/µL) of DNA sample. The PCR reaction included an initial denaturation of 95 °C for 5 minutes, followed by 40 cycles of 95 °C for 5 minutes, 50 °C for 30 seconds and 72 °C for 30-60 seconds, and a final extension at 72 °C for 4 minutes. All PCR fragments were separated using 1.5% agarose gel electrophoresis stained with safe view™ classic dye (1ml, G108, abm) and electrophoresed at 100V for 40 minutes using Quick load 100 bp DNA ladder (New England, Biolabs) was used as a molecular marker and visualized using UV light excitation. Image was digitalized using a Gel Documentation System (Nagar, India).

#### Data analysis

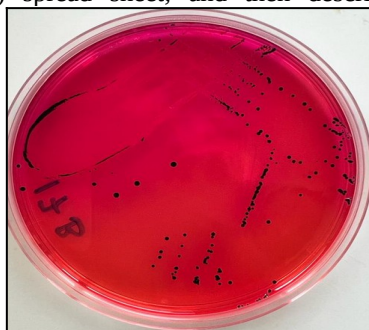
The data were entered into Microsoft Excel (2016) spread sheet, and then descriptive

analysis was performed using the R statistical software 4.3.1. The data on the prevalence of poultry type samples (broilers, layers and *backyard* chickens) were examined using the Chi-square test, in all cases, a p-value of less or equal to 0.05 was considered statistically significant.

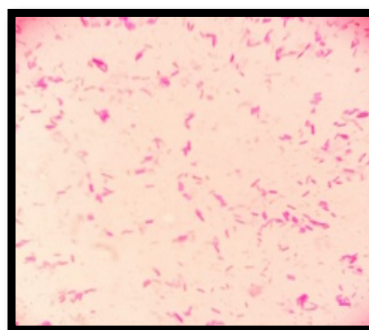
## Results

### Salmonella Isolation and Biochemical Identification

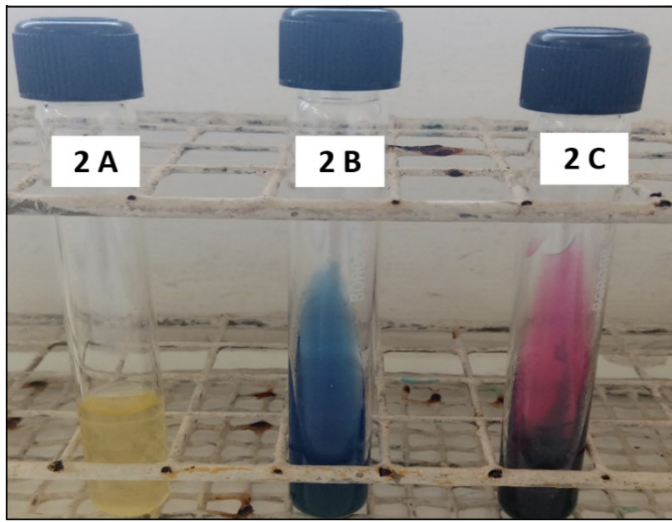
Red colonies with black spot at the center on XLD medium were presumed to be *Salmonella* (Figure 1). Under microscope *Gram* negative rods was seen using 100X objective lens (Figure 2) In biochemical tests *Salmonella* isolates tested urease negative (Figure 3 (2A)), citrate negative (Figure 3 (2B)). TSI tested positive for gas production by forming cracks and positive for hydrogen sulfide gas by forming black colour, red slant (alkaline) and butt yellow (Acidic) (Figure 3 (2C)).



**Figure 1:** XLD Agar Plate Showing the Growth of Presumptive *Salmonella* with Red Colonies with Black Spot at the Center



**Figure 2:** Gram negative rods bacteria under microscope



**Figure 3:** Biochemical Identification of *Salmonella*; 2A- Urease Negative, 2B- Citrate Positive and 2C- TSI Positive.

The prevalence of *Salmonella* in poultry was 8% (N=400). The prevalence of *Salmonella* infection at district level is shown (Table 3). The number of broilers infected with *Salmonella* was significantly higher compared with that of layers and backyard chickens ( $P = 0.037$ ) (Table 4).

**Table 2:** Prevalence of NTS in poultry from each district in Dar es Salaam.

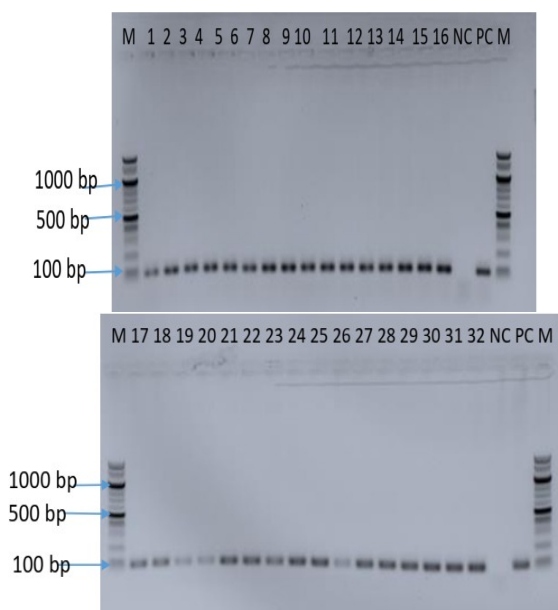
| Districts | Area       | Positive  | Negative   | Total      |
|-----------|------------|-----------|------------|------------|
| All       | Samples    | 32 (8%)   | 368 (92%)  | 400 (100%) |
| Ubungo    | Shekilango | 4 (13.3%) | 26 (86.6%) | 30 (100%)  |
|           | Manzese    | 4 (13.3%) | 26 (86.6%) | 30 (100%)  |
|           | Farmers    | 2 (5%)    | 38 (95%)   | 40 (100%)  |
| Ilala     | Kisutu     | 5 (16.6%) | 25 (83.3%) | 30 (100%)  |
|           | Buguruni   | 3 (10%)   | 27 (90%)   | 30 (100%)  |
|           | Farmers    | 0 (0%)    | 40 (100%)  | 40 (100%)  |
| Temeke    | Stereo     | 4 (13.3%) | 26 (86.6%) | 30 (100%)  |
|           | Zakhem     | 2 (6.6%)  | 28 (93.3%) | 30 (100%)  |
|           | Farmers    | 1 (2.5%)  | 39 (97.5%) | 40 (100%)  |
| Kinondoni | Magomeni   | 3 (10%)   | 27 (90%)   | 30 (100%)  |
|           | Mtambani   | 2 (6.6%)  | 28 (93.3%) | 30 (100%)  |
|           | Farmers    | 2 (5%)    | 38 (95%)   | 40 (100%)  |

**Table 3:** Number of chickens infected with *Salmonella* isolates from each district

| Location  | Type of poultry |          |          | Total | Mean | Standard deviation |
|-----------|-----------------|----------|----------|-------|------|--------------------|
|           | layers          | Broilers | Backyard |       |      |                    |
| Kinondoni | 1               | 4        | 2        | 7     | 2.3  | 1.5                |
| Ubungu    | 2               | 6        | 2        | 10    | 3.3  | 2.3                |
| Temeke    | 2               | 4        | 1        | 7     | 2.3  | 1.5                |
| Ilala     | 4               | 4        | 0        | 8     | 2.7  | 2.3                |
| Total     | 9               | 18       | 5        |       |      |                    |
| Mean      | 2.25            | 4.5      | 1.2      |       |      |                    |

#### PCR-Confirmatory Identification of *Salmonella* isolates.

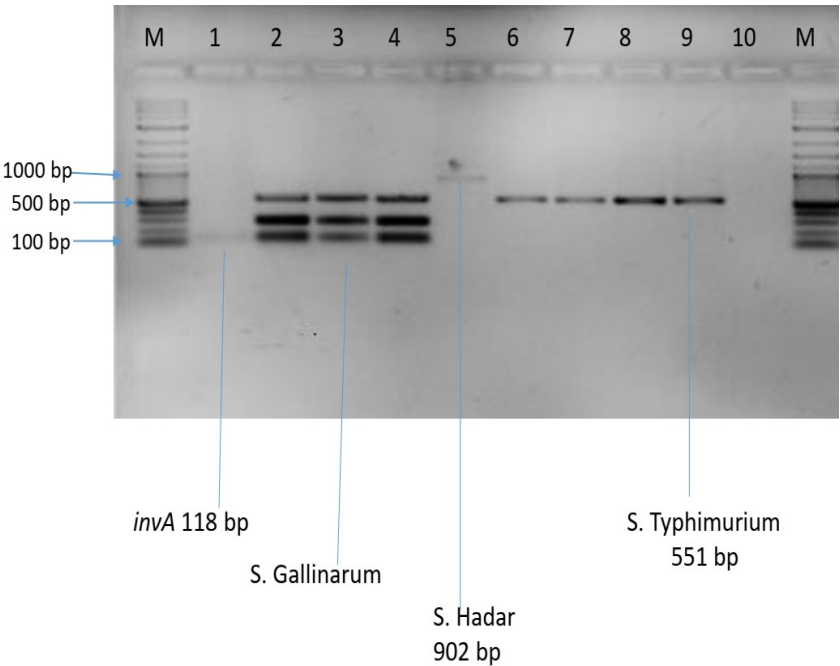
All 32 *Salmonella* isolates were confirmed by amplifying *Salmonella* specific gene (*invA*) of 118 bp (figure 4).



**Figure 4:** Gel Electrophoresis Image Showing *Salmonella* Gene Detection Based on *invA* gene amplification (Expected band size -118bp). M= DNA Marker (100bp), NC= Negative Control, PC=Positive Control, Lane (1, 2, 3, 5, 20) = Backyard Chicken, Lane (4, 6, 9, 10, 11, 12, 13, 14, 16, 17, 19, 21, 22, 25, 27, 28, 31 and 32) = Broiler and Lane (7, 8, 15, 18, 23, 24, 26, 29 and 30) = Layers

#### PCR-Based Determination of *Salmonella* Serovars

Three out of seven targeted serovars were detected by PCR among 32 *Salmonella* isolates (Figure 5).



**Figure 5:** Gel Electrophoresis Image representing all *Salmonella* serovars detected. Lane M: DNA marker (100 bp), lane 1: *invA* (118 bp), lane 2- 4: Representing multiplex PCR for *S. Gallinarum*, lane 5: *S. Hadar* (902 bp), lane 6-9: *S. Typhimurium* (551 bp) and lane 10: unidentified isolate.

The distribution of NTS serovars in layers, broilers and backyard chickens are shown (Table 5). The most prevalent serovar was *S. gallinarum* and serovar of one (1) *Salmonella* isolate from broiler chickens, could not be determined by the primers used.

**Table 5:** Distribution of NTS serovars in the poultry flocks

| NTS Serovars          | Type of poultry |          |          | Total     |
|-----------------------|-----------------|----------|----------|-----------|
|                       | Layers          | Broilers | Backyard |           |
| <i>S. hadar</i>       | 1 (100%)        | 0 (0%)   | 0 (0%)   | 1 (3%)    |
| <i>S. typhimurium</i> | 2 (40%)         | 1 (20%)  | 2 (40%)  | 5 (16%)   |
| <i>S. gallinarum</i>  | 6 (28%)         | 16 (60%) | 3 (12%)  | 25 (78%)  |
| <b>Total</b>          | 9 (29%)         | 17 (55%) | 5 (16%)  | 31 (100%) |

### Discussion

In this study 8% of healthy broiler, layer and backyard chickens were confirmed to be infected with NTS. The NTS prevalence in Dar es Salaam was higher compared to earlier reports (García et al. 2011, Munuo et al. 2022). On the other hand, the burden of NTS in the current work was lower compared to

NTS prevalence reported from South-Central Peninsular Malasia, Nigeria, Dhaka and South Africa (Karim et al. 2017, Fagbamila et al. 2017, Jajere et al. 2019, Ramtahal et al. 2022), while the current finding was closely similar to NTS prevalence reported previously (Kalaba et al. 2017). Such variations in the prevalence could be

attributed by several factors including; sample sizes, sampling technique, methods used for isolation, age of chicken, poultry housing and weather conditions (Mdemu et al. 2016, Ramtahal et al. 2022).

The current study indicated that the NTS burden was significantly higher in broiler chickens compared to that of layer and backyard chickens. While there was no significant difference in NTS burden between layer and backyard chickens. This result is in line with previous finding reported by Sharma et al. (2021). Broilers are more contaminated than layers and backyard chickens because they are fast growing and are kept in higher stocking densities (intensive rearing) which led to poor management including difficult in cleaning and disinfection of broiler houses or cages hence increase the chance of contamination and transmission (Sharma et al. 2021). Furthermore, poor biosecurity practices, feeds and water contamination contribute to infection among poultry (Mdemu et al. 2016, Fagbamila et al. 2017).

On the other hand, even though backyard chickens had found to have low prevalence of *Salmonella* compared to *broilers* and layers, but other studies have shown that they are more likely to be infected than broilers and layers because they are randomly kept during the day this increase the probability/chance of being contaminated due to direct contact with transmitting vectors such as insects, rodents and other environmental factors (Kindu and Addis 2013, Munuo et al. 2022).

In this study three known serovars were detected, namely *S. hadar* (3%), *S. typhimurium* (16%) and *S. gallinarum* (78%). High prevalence of *S. gallinarum* has been reported elsewhere (Gong et al. 2014, Zhou et al. 2022). *S. gallinarum* biovars (*S. pullorum* and *S. gallinarum*) are causative agents of pullorum and fowl typhoid diseases respectively and they have been outlined by World Organization for Animal Health as the more important economic diseases in poultry industry (Xiong et al. 2018, Zhou et al. 2022). The present study report *S. hadar* at 3% which agrees with the study by Ferrari et al. (2019). *S. hadar* is among the serovars

which causes salmonellosis outbreaks in Africa and it is of much concern because of its potential for multidrug resistance (MDR) (Ferrari et al. 2019). *S. hadar* has also been reported to cause diarrhoea in humans including infants in Germany (Deshpande et al. 2015, Ye et al. 2021).

Although *S. enteritidis* and *S. typhimurium* are the most predominant NTS serovars reported globally (Siddiky et al. 2022), the current study did not detect *S. enteritidis* but detected low prevalence of *S. typhimurium* (16%). However, in developed countries the reported decrease in *S. typhimurium* and *S. enteritidis* was associated with the use of vaccines against them (Kalaba et al. 2017). The current study also did not detect *S. thompson* and *S. infantis*, failure to detect some NTS serovars targeted might be due to the fact that the epidemiology of NTS serovars in food differ according to countries production practices and geographical locations (Ferrari et al. 2019, Peruzzy et al. 2022)

## Conclusion

The findings from this study confirmed the presence of NTS in healthy chickens, *S. gallinarum* which infect poultry only and (*S. typhimurium* and *S. hadar*) which infect both humans and animals, this confirms that the humans and chickens' health are in danger as these serovars have been reported to cause salmonellosis. The study revealed the presence of important NTS in poultry population that can cause diseases in humans and animals in Dar es Salaam poultry farms. This calls for animal and health authorities to design and plan participatory health education that will address poultry farm biosecurity, poultry market environment hygiene and poultry carcass dressing. This will improve poultry meat safety and minimize the likelihood of salmonellosis outbreaks. Also, more researches and surveillance are needed to monitor the NTS strains circulating in poultry in order to come up with appropriate mitigation measures including development of combined local vaccines against important NTS serovars which infect poultry.

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## Conflict of interests

The authors declare that there is no conflict of interests.

## Ethics approval and consent to participate

Permission to conduct this research was granted by the Tanzania Livestock Research Institute (TALIRI) Ethics Review Committee (Ref. No. TLRI/CC.21/044).

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