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**Original Research Article**

# Prevalence of multidrug resistant *Salmonella* Typhimurium in retailed buffalo meat and offal with a reduction trial using rosemary and olive oils

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**Abstract**

The objectives of the current study were first to investigate the prevalence rates of *Salmonella* spp. in the buffalo meat and edible offal (round, masseter muscles, liver, kidney, and trimmings) retailed in Zagazig city, Egypt. Second, serological identification of the isolated *Salmonella* spp., was followed. Third, screening of antimicrobial sensitivity testing of the identified *Salmonella* Typhimurium was done using the disk diffusion assay. Finally, the inhibitory effects of rosemary and olive oils against *Salmonella* Typhimurium were investigated. The obtained results in the present study revealed isolation of *Salmonella* spp., from the examined round, masseter muscles, liver, kidney, and trimmings at 15%, 25%, 35%, 25%, and 50%, respectively. Serological identification of Salmonella spp. revealed recovery of six serotypes namely, *S.* Typhimurium, *S.* Enteritidis, *S.* Kentucky, *S.* Inganda, *S.* Apeyeme, and *S.* Anatum from the examined samples at variable rates. The overall isolation rates of these serotypes were 26.64%, 29.97%, 16.65%, 9.99% 9.99%, and 6.66%, respectively. *Salmonella* Typhimurium isolates had clear multidrug resistance profiles. Rosemary and olive oils at 0.1%, and 0.5% could significantly reduce *S.* Typhimurium in an experimental trial in a concentration-dependent phenomenon.

***Keywords:*** Buffalo meat; offal; *Salmonella* spp.; rosemary; olive oils

## Introduction

Buffalo meat and edible offal are considered as essential sources of animal-derived protein with high biological values, vitamins such as vitamin B group, and minerals such as zinc, iron, and selenium. Buffalo meat has almost the same characteristics of the beef, therefore, it is regarded as an important alternative for beef in many parts of the world **(Cockrill, 1981; Preiato, 2020; Tang et al., 2020)**. However, buffalo meat and offal might be considered as potential source of foodborne pathogens such as *Listeria monocytogenes*, *E. coli*, *Citrobacter spp.*, *Staphylococcus aureus (Staph aureus)*, and *Salmonella* spp., **(Hassan et al., 2001; Saud et al., 2019)**.

Microbial contamination of buffalo meat and edible offal such as masseter muscle, liver, kidney, and trimmings might take place during any step of processing starting from the act of slaughter, skinning, evisceration, distribution, and storage **(Liu et al., 2020)**.

Among foodborne pathogens, *Salmonella* spp., was frequently associated with meat contamination and human illness worldwide **(Bantawa et al., 2019)**. Consumption of *Salmonella*-contaminated foods was reported to cause 3 million deaths annually **(Goburn et al., 2007)**. The clinical symptoms of *Salmonella* infection include typhoid fever, enteritis, and bacteremia **(Santos et al., 2001)**. Non-typhoid Salmonellosis has been linked with acute gastroenteritis with unpleasant effects on the surrounding organs **(Su et al., 2004)**.

The continuous and uncontrolled usage of antimicrobials during livestock production had led to the development of the drug resistance phenomenon among the originated foodborne pathogens **(Darwish et al., 2013)**. However, the role of the buffalo

meat and edible offal as potential sources of multidrug resistant *Salmonella* spp., in Egypt has received less attention.

The use of natural food additives in the meat industry is increased worldwide for the purposes of providing attractive colors, aroma, flavor, and as antimicrobials. Among these, rosemary (*Rosmarinus officinalis L.*) essential oil has been used in the meat industry for its antimicrobial activities against several food poisoning microorganisms such as *Escherichia coli*, and *Bacillus cereus* **(Chraibi et al., 2020)**. Besides, olive oil had significant *in vitro* antimicrobial effects, particularly against *Staph. aureus*, *and Salmonella* Typhimurium **(Guo et al., 2020)**.

This study was done to study the prevalence rates of Salmonella *spp*., particularly *Salmonella* Typhimurium, in the retailed buffalo meat and edible offal (round, masseter muscles, liver, kidney, and trimmings) in Egypt. Furthermore, detection of the antimicrobial sensitivity of the identified *Salmonella* Typhimurium was done using the disk diffusion assay. In addition, the inhibitory effects of rosemary and olive oils against *Salmonella* Typhimurium were examined.

## Material and Methods

*Collection of samples:*

A hundred random samples including 20 each of round, masseter muscles, liver, kidney, and trimmings were collected from butchery shops at different sanitation levels in Zagazig city, Egypt. Samples were moved directly to the laboratory for microbiological examination.

*Sample preparation:*

Samples were prepared according to the guidelines of **APHA (2001)**. In brief, ten grams from each sample were homogenized in 90 ml of 1% sterile peptone water (Oxoid CM9).

*Isolation and identification of Salmonella* spp.

Salmonella isolation, and identification were done according to **ISO 6579 (2002)**. In short, ten ml of the prepared homogenate were incubated at 37°C for 18 ± 2 h as pre-enrichment procedure. Selective enrichment was done on Rappaport Vassiliadis with soya broth at 41.5°C for 24 ± 2 h. A loopful from the enriched culture was streaked on the surface of xylose lysine desoxycholate (XLD) agar plate and incubated 37°C for 24 ± 2 h. Suspected colonies (non-lactose fermenters) were red with or without black centers. Such colonies were purified and sub-cultured onto nutrient agar slopes and incubated at 37°C for 24 h. The purified colonies were subjected to morphological, biochemical, and serological identification.

*Antibiogram of the identified Salmonella Typhimurium:*

Antimicrobial sensitivity testing of the recovered isolates of *Salmonella Typhimurium* was tested using the disk diffusion method. Antimicrobial discs were purchased from Oxoid Limited, Hampshire, UK. Nutrient agar plates acted as a culture medium for *Salmonella Typhimurium*. The guidelines of the **National Committee for Clinical Laboratory Standards (NCCLS, 2001)** were applied. Multiple Antibiotic Resistance (MAR) index for each strain was determined according to the formula stipulated by **Singh *et al.* (2010)** as follow:

MAR index= No. of resistance / Total No. of tested antibiotics

The tested antimicrobials were ampicillin (AMP), cephalothin (CN), chloramphenicol (CH), ciprofloxacin (CP), enrofloxacin (EN), erythromycin (E), gentamicin (G), kanamycin (K), nalidixic acid (NA), neomycin (N), oxacillin (OX), oxytetracycline (T), Streptomycin (S), and sulfamethoxazole (SXT).

*An experimental trial to investigate the inhibitory effects of rosemary and olive oils against* *Salmonella* Typhimurium*.:*

The antibacterial effects of rosemary and olive oils (National Research Center, Dokki, Giza, Egypt) were tested at two concentrations (0.1, and 0.5%). Muscle samples (1.5 kg free from fat) were divided into 15 pieces (each piece is 100 g). Then 12 pieces were artificially inoculated with *Salmonella* Typhimurium according to **Govaris et al. (2010)**. After that, five groups were planned (n = 3/group). Group 1 was acted as a control, where samples were not inoculated with the tested bacterium. Group 2 was treated with rosemary oil 0.1%, group 3 was treated with rosemary oil 0.5%, group 4 was treated with olive oil 0.1%, and group 5 was treated with olive oil 0.5%. Treatment groups were soaked in the tested oils for 30 min at room temperature and examined for microbiological counts.

*Organoleptic examination:*

It was carried out according to **Pearson and Tauber (1984)**. The overall acceptability was based on the color, odor, and consistency.

*Statistical analysis:*

All microbial counts were transferred into base-10 logarithms of CFU/g. Data were analyzed using one-way ANOVA procedure of SPSS v.23 (SPSS Inc., Chicago, Illinois,

The USA). Tukey's multiple comparison tests were used to test significant variations. Data were expressed as means ± SD, with a *P*-value of 0.05 is considered significant.

**Results and Discussion**

The obtained results in the present study revealed isolation of *Salmonella* spp., from the examined round, masseter muscles, liver, kidney, and trimmings at 15%, 25%, 35%, 25%, and 50%, respectively **(Fig. 1)**.

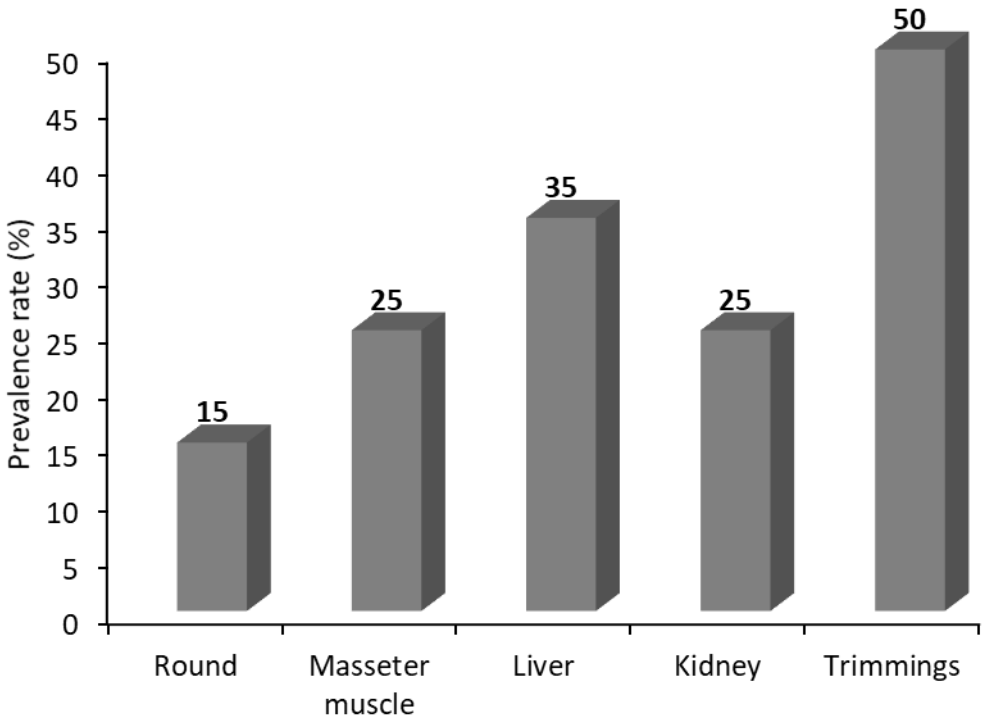



*Fig. 1: Prevalence rates (%) of Salmonella spp. in the examined buffalo meat and edible offal*

Further serological identification of the isolated *Salmonella* spp. revealed recovery of six serotypes namely, *S.* Typhimurium, *S.* Enteritidis, *S.* Kentucky, *S.* Inganda, *S.* Apeyeme, and *S.* Anatum from the examined samples at variable rates. The overall

isolation rates of the aforementioned serotypes were 26.64%, 29.97%, 16.65%, 9.99% 9.99%, and 6.66%, respectively. *S.* Typhimurium was isolated at 3.33% from each of masseter muscles, and kidney; and at 9.99% from liver, and trimmings. *S.* Enteritidis had the highest prevalence rate as it was isolated at 3.33% from each of round, masseter muscles, and liver; and at 6.66% from kidney, and at 13.32% from trimmings. The other identified serotypes were isolated at lesser rates **(Table 1)**. The obtained results go in agreement with **Bantawa et al. (2019)** who isolated *Salmonella* spp., from buffalo, pork, and goat meats collected from Nepal at 35%. While in another study from the same country **Saud et al. (2019)** isolated *Salmonella* spp., from buffalo meat at 7.4%. Higher isolation rates was reported by **Boonmar et al. (2013)** who isolated *Salmonella* spp., from buffalo meat retailed in Pakse, Champasak Province, Laos at 80%. The most prevalent Salmonella serotypes were *S.* Stanley, *S.* Anatum, *S.* Derby, *S.* Rissen, and *S.* Amsterdam. Unlikely, lower isolation rate was reported in the frozen trimmings collected from Indian buffalo at 0.87% in India **(Biswas et al., 2008)**. This variation in the isolation rates of Salmonella might be attributed to the sanitation level and hygienic practices adopted during the preparation of buffalo meat and offal.

*Salmonella* Typhimurium had clear multidrug resistance profile as declared in **Fig. 2, and Table 2**. The antimicrobial resistance profile of the recovered *S.* Typhimurium was as following: ampicillin (12.5%), cephalothin (25%), chloramphenicol (25%), ciprofloxacin (37.5%), enrofloxacin (25%), erythromycin (100%), gentamicin (25%), kanamycin (37.5%), nalidixic acid (100%), neomycin (25%), oxacillin (37.5%), oxytetracycline (50%), Streptomycin (25%), and sulfamethoxazole (75%). *Salmonella* Typhimurium had an

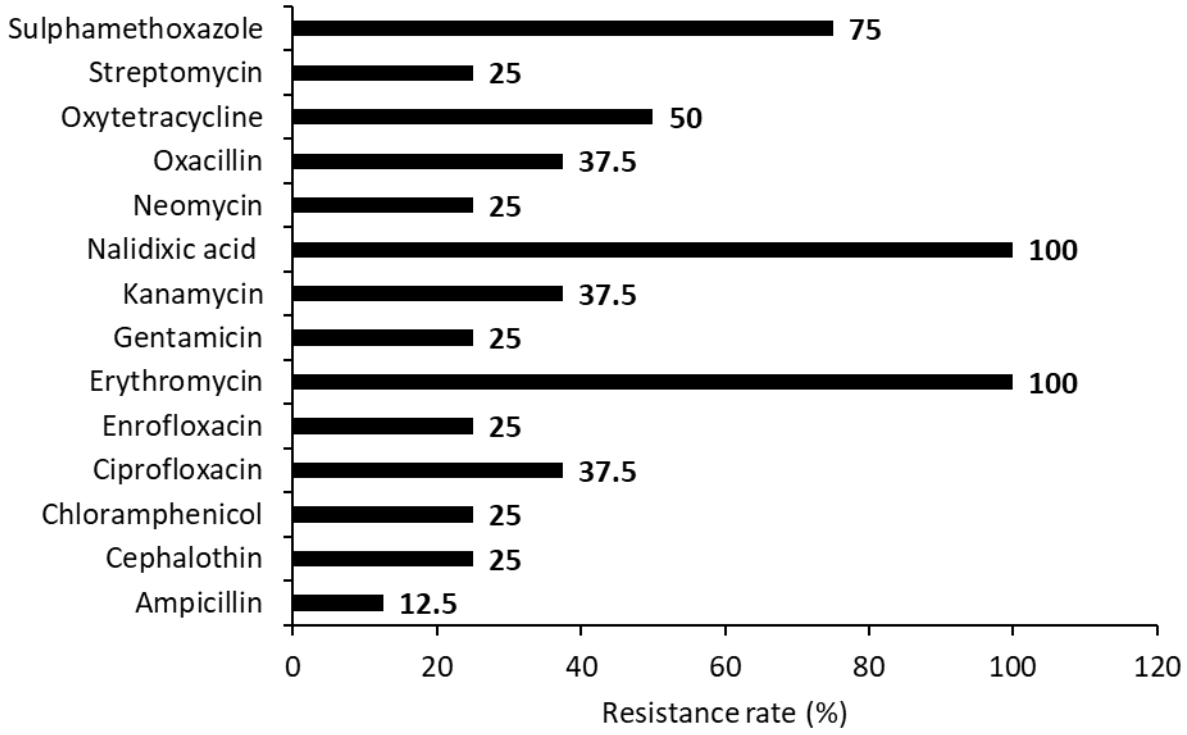
163 average MAR index of 0.429. The obtained results were comparable to that reported
164 everywhere. For instances, *Salmonella* spp., isolated from retailed buffalo meat in Laos
165 were resistant to streptomycin (67%), tetracycline (67%), and ampicillin (63%). Of the
166 isolates, 73% were multidrug-resistant **(Boonmar et al., 2013)**. Besides, *Salmonella* spp.
167 isolated from buffalo meat in Nepal were resistant to amoxicillin, tetracycline,
168 cotrimoxazole and nalidixic acid with 21.9% of the isolates had multidrug resistance profile
169 **(Saud et al., 2019)**.

170 There are continuous efforts to find alternatives to the chemical preservatives with
171 antimicrobial activities. In this regard, a trial to investigate the inhibitory effects of
172 rosemary and olive oils against *S.* Typhimurium. Interestingly, the two used oils had
173 significant inhibitory effects against *S.* Typhimurium in a concentration-dependent manner
174 **(Fig. 3)**. Rosemary oil at 0.1%, and 0.5% concentrations reduced *S.* Typhimurium at
175 12.59%, and 24.14%, respectively, whereas olive oil at 0.1%, and 0.5% concentrations
176 reduced *S.* Typhimurium at 9.58%, and 25.76%, respectively. At the same time, the used
177 oils did not change the sensory characteristics (brick red color, firm in consistency, and
178 fresh odor) of the round muscle at the two tested oil concentrations (0.1%, and 0.5%) (Data
179 are not shown). In agreement with these findings, rosemary oil had clear antibacterial
180 activities against S. *S.* Enteritidis, and *S.* Typhi **(Bozin et al., 2007)**. Besides, olive oil
181 polyphenolic extracts inhibited the growth of S. Typhimurium and *Staph. aureus* at 0.625
182 mg/mL for 3 hours incubation, and 0.625-1.25 mg/mL for 5 hours incubation, respectively
183 using *in vitro* approaches **(Guo et al., 2020)**. The proposed mechanisms for the
184 antimicrobial effects of the examined essential oils involved loss of the mitochondrial

185 membrane in the bacteria, coagulation of the cellular proteins, and affecting the proton
186 pump and ion channels **(Tariq et al., 2019)**.
187

188 **Table 1: Prevalence and antigenic structure of the isolated *Salmonella* serotypes from buffalo meat and edible offal**

| ***Salmonella* serotypes** | **Round (n=3)** | | **Masseter muscle (n=5)** | | **Liver (n=7)** | | **Kidney (n=5)** | | **Trimmings (n=10)** | | **Total (n=30)** | | **Group** | **Antigenic Structure** | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **No.** | **%** | **No.** | **%** | **No.** | **%** | **No.** | **%** | **No.** | **%** | **No.** | **%** | | **O** | **H** |
| *S.* Typhimurium | 0 | 0 | 1 | 3.33 | 3 | 9.99 | 1 | 3.33 | 3 | 9.99 | **8** | **26.64** | B | 1, 4, 5, 12 | i: 1,2 |
| *S.* Enteritidis | 1 | 3.33 | 1 | 3.33 | 1 | 3.33 | 2 | 6.66 | 4 | 13.32 | **9** | **29.97** | D | 1, 9, 12 | g, m: - |
| *S.* Kentucky | 1 | 3.33 | 1 | 3.33 | 2 | 6.66 | 0 | 0 | 1 | 3.33 | **5** | **16.65** | E1 | 8, 20 | i: $Z_6$ |
| *S.* Inganda | 0 | 0 | 1 | 3.33 | 0 | 0 | 1 | 3.33 | 1 | 3.33 | **3** | **9.99** | C1 | 6, 7 | Z10: 1,5 |
| *S.* Apeyeme | 1 | 3.33 | 1 | 3.33 | 0 | 0 | 1 | 3.33 | 0 | 0 | **3** | **9.99** | C3 | 8, 20 | Z38: - |
| *S.* Anatum | 0 | 0 | 0 | 0 | 1 | 3.33 | 0 | 0 | 1 | 3.33 | **2** | **6.66** | E1 | 3, 10 | e, h: 1,6 |
| **Total** | **3** | **9.99** | **5** | **16.65** | **7** | **23.31** | **5** | **16.65** | **10** | **33.3** | **30** | **100** | | | |

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190 **Fig. 2: Antibiogram of the recovered *S.* Typhimurium**

191

192 **Table 2: Antimicrobial resistance pattern among the isolated S. Typhimurium strains**
193 **from buffalo meat and edible offal**

| ***S.* Typhimurium strain** | **Resistant antimicrobial** | **MAR index** |
|---|---|---|
| *S.* Typhimurium 1 | AMP, CN, CH, CP, EN, E, K, NA, N, OX, T, S, SXT | 0.928 |
| *S.* Typhimurium 2 | CN, CH, CP, EN, E, K, NA, N, OX, T, S, SXT | 0.857 |
| *S.* Typhimurium 3 | CP, E, G, K, NA, OX, T, SXT | 0.571 |
| *S.* Typhimurium 4 | E, G, NA, T, SXT | 0.357 |
| *S.* Typhimurium 5 | E, NA, SXT | 0.214 |
| *S.* Typhimurium 6 | E, NA, SXT | 0.214 |
| *S.* Typhimurium 7 | E, NA | 0.143 |
| *S.* Typhimurium 8 | E, NA | 0.143 |
| | **Average** | **0.429** |

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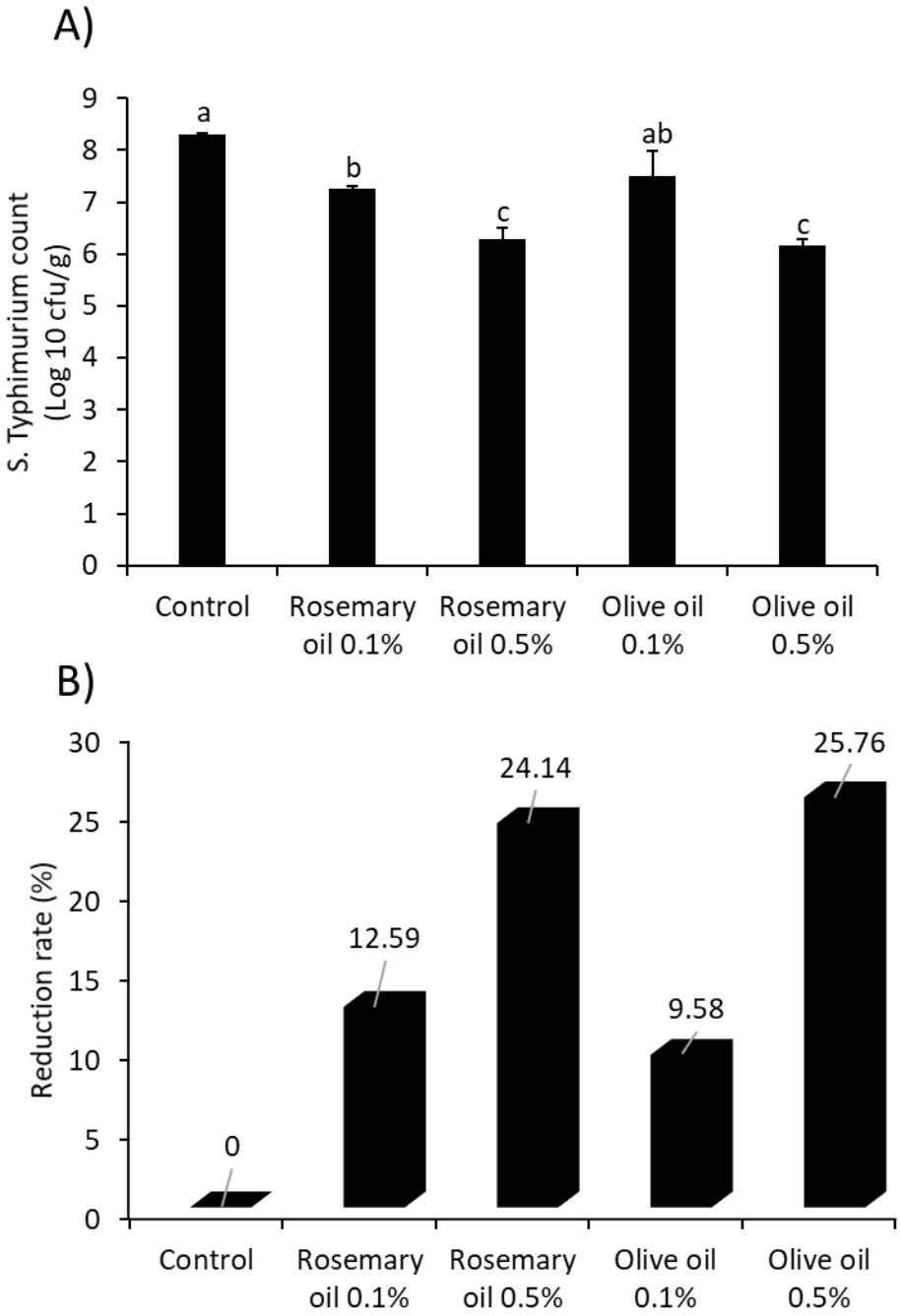


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197 *Fig. 3: A) Effects of rosemary and olive oils on S. Typhimurium count (Log 10 cfu/g) in*
198 *buffalo round experimentally inoculated with S. Typhimurium B) The reduction rates (%) of*
199 *rosemary and olive oils against S. Typhimurium. Columns with different letter (a, b) are*
200 *significantly different at $p < 0.05$.*

201

## Conclusion

The obtained results in the current investigation revealed isolation of multidrug resistant *Salmonella* spp., particularly S. Typhimurium from retailed the examined buffalo meat and edible offal at variable rates. This indicates unsatisfactory hygienic measures adopted during slaughtering, evisceration, and processing of buffalo carcasses. Therefore, strict hygienic procedures should be followed in slaughterhouses and butchery shops. In addition, using of rosemary and olive oils at 0.1%, and 0.5% had significant inhibitory effects against *S.* Typhimurium in an experimental trial.

**Conflict of interest:** None

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