Prevalence of multidrug resistant *Salmonella Typhimurium* inretailed buffalo meat and offal with 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

1. 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 *Salmonella* isolates 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* (Chrabti 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.

2. Materials and Method

2.1. 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.

2.2. 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).

2.3. 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.

2.4. Antibiotic sensitivity 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:

\[ \text{MAR index} = \frac{\text{No. of resistance}}{\text{Total No. of tested antibiotics}} \]
The tested antimicrobials were ampicillin (AMP), cephalexin (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).

2.5. 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 prominent Salmonella serotypes were S. Typhimurium. Besides, Salmonella spp. isolated from buffalo meat in Nepal were inoculated. Unlikely, the two used oils had significant inhibitory effects against S. Typhimurium in a concentration-dependent manner (Fig. 3). Rosemary oil at 0.1%, and 0.5% concentrations reduced S. Typhimurium at 12.59%, and 24.14%, respectively, whereas olive oil at 0.1%, and 0.5% concentrations reduced S. Typhimurium at 9.58%, and 25.76%, respectively. At the same time, the used oils did not change the sensory characteristics (brick red color, firm in consistency, and fresh odor) of the round muscle at the two tested oil concentrations (0.1%, and 0.5%) (Data are not shown). In agreement with these findings, rosemary oil had clear antibacterial activities against S. S. Enteritidis, and S. Typhi (Bozin et al., 2007). Besides, olive oil polyphenolic extracts inhibited the growth of S. Typhimurium and Staph. aureus at 0.625 mg/mL for 3 hours incubation, and 0.625-1.25 mg/mL for 5 hours incubation, respectively using in vitro approaches (Guo et al., 2020). The proposed mechanisms for the antimicrobial effects of the examined essential oils involved loss of the mitochondrial membrane in the bacteria, coagulation of the cellular proteins, and affecting the proton pump and ion channels (Tariq et al., 2019).

4. Conclusion

The obtained results in the current investigation revealed isolation of multidrug resistant Salmonella spp., particularly S. Typhimurium from retail of the examined buffalo meat and edible offal at variable rates. This indicates unsatisfactory hygienic measures adopted during slaughtering, visceraion, 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.

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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. | % | No. | % |
| 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 | 5, 7, 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: Z6 |
| S. Aganda            | 0   | 0 | 1   | 3.33 | 0   | 0   | 1   | 3.33 | 1   | 3.33 | 3   | 9.99 | C1 | 6, 7 | Z2:i, 1.5 |
| S. Apeyeme           | 1   | 3.33 | 1   | 3.33 | 0   | 0   | 1   | 3.33 | 0   | 0   | 3   | 9.99 | C3 | 8, 20 | Z18: |
| S. Anatum           | 0   | 0 | 0   | 0   | 1   | 3.33 | 0   | 0   | 1   | 3.33 | 2   | 6.66 | E1 | 3, 19 | e, H: 1.8 |
| Total                | 3   | 9.99 | 5   | 16.65 | 7   | 23.31 | 5   | 16.65 | 10  | 33.3 | 30  | 100   |

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

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