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

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A B S T R A C T

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 Salmonellosis has been linked with acute gastroenteritis with unpleasant effects on the surrounding organs (Su et al., 2004).

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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.

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).

2.3.0Isolation 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. 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).

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 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.

2.6. Organoleptic examination:

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

2.7. 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 significan.

3. 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).

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

There are continuous efforts to find alternatives to the chemical preservatives with antimicrobial activities. In this regard, a trial to investigate the inhibitory effects of rosemary and olive oils against S. Typhimurium. Interestingly, 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 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

5. References

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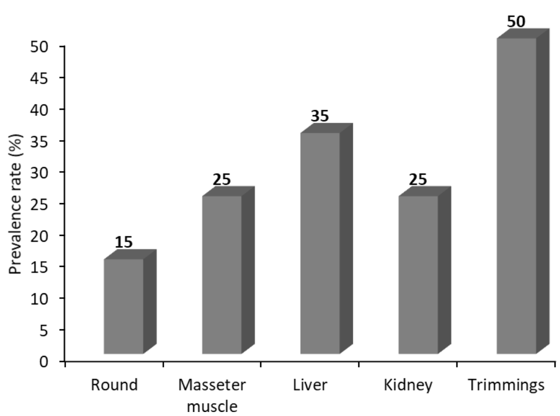

Fig. 1: Prevalence rates (%) of Salmonella spp. in the examined buffalo meat and edible offal

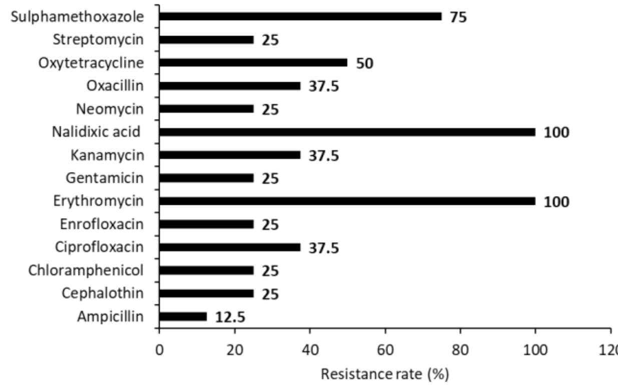

Fig. 2: Antibiogram of the recovered S. Typhimurium

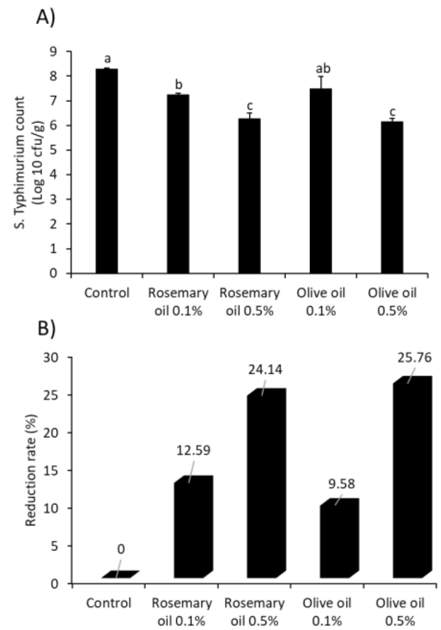

Fig. 3: A) Effects of rosemary and olive oils on S. Typhimurium count (Log 10 cfu/g) in buffalo round experimentally inoculated with S. Typhimurium B) The reduction rates (%) of rosemary and olive oils against S. Typhimurium. Columns with different letter (a, b) are significantly different at $p < 0.05$.

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			

Table 2: Antimicrobial resistance pattern among the isolated S. Typhimurium strains 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