PREVALENCE AND ANTIBIOGRAM OF *Listeria monocytogenes* IN RETAILED BUFFALO RAW MEAT AND MINCE WITH A PROTECTION TRIAL USING NISIN, AND GINGEROL

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ABSTRACT

Buffalo meat is an emerging source of high-quality animal protein. However, the role of the buffalo meat in the transmission of foodborne pathogens such as *Listeria monocytogenes* (*L. monocytogenes*) is scarcely investigated. Therefore, this study aimed at investigation of the prevalence rates of *Listeria* spp., particularly *L. monocytogenes* in retailed buffalo meat and buffalo mince in the Egyptian markets. In addition, antimicrobial susceptibility testing of the recovered *L. monocytogenes* isolates was further screened. Furthermore, antilisterial effects of two natural food additives, namely nisin, and gingerol were further examined. The obtained results of the present study revealed an overall isolation rates of *Listeria* spp. and *L. monocytogenes* from all examined samples at 34%, and 10%, respectively. Serological identification of the isolated *Listeria* spp. revealed recovery of six *Listeria* spp. namely, *L. ivanovii*, *L. welshimeri*, *L. innocua*, *L. seeligeri*, *L. grayi*, and *L. monocytogenes. L. monocytogenes* was isolated at 6%, and 14% from the examined buffalo meat and buffalo mince, respectively. The recovered *L. monocytogenes* had multidrug resistance profiling. Nisin and gingerol had clear antilisterial activities. As nisin achieved reduction rates of 11.36%, and 44.84% at 1%, and 2%, respectively; while gingerol achieved reduction rates of 8.44%, and 36.17% at 1%, and 2%, respectively. Therefore, it is recommended to use such food additives for the control of *L. monocytogenes* in the meat industry.

Keywords: *Bubalus bubalis*, buffalo meat, listeria monocytogenes, multidrug resistance, nisin, gingerol

INTRODUCTION

Buffalos' farming has recently increased worldwide, particularly in Egypt, and central Asian countries. Buffalo meat is considered as a rich

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source of high-quality animal protein, vitamins such as vitamin B1, B6, and B12, and minerals such as zinc, selenium, and iron (Cockrill, 1981; Preiato, 2020). However, the possible contribution of buffalo meat and meat products in the spread of foodborne pathogens has gained less attention.

Listeria species are Gram's positive, facultative intracellular organisms. They are ubiquitous organisms with prominent abilities to grow over a wide range of temperatures and food substrates (Weller *et al*., 2015). *Listeria monocytogenes* (*L. monocytogenes*) has emerged as a foodborne pathogen with zoonotic importance. It causes human listeriosis, which causes symptoms including gastroenteritis, fever, chills, abortion, meningitis, and encephalitis, and might lead to death (Abdel-Malek *et al*., 2010; Dimic *et al*., 2010). The uncontrolled usage of antimicrobials during livestock production has resulted in the development of multidrug resistance among the foodborne pathogens (Darwish *et al*., 2013). Multidrug resistant *L. monocytogens* has become a critical health issue worldwide (Barbuddhe *et al*., 2002). Inadequate hygienic precautions followed during processing, handling, storage, and distribution of meat and meat products might lead to their contamination with foodborne pathogens such as *L. monocytogenes* (De Cesare *et al*., 2017; Liu *et al*., 2020). *L. monocytogenes* was isolated from different food matrices such as sea foods (Vongkamjan *et al*., 2017), cattle meat and meat products (Matle *et al*., 2020), and vegetables (Maćkiw *et al*., 2021). However, there is limited information available about the prevalence of multidrug resistant *L. monocytogenes* in buffalo meat and meat products, particularly in Egypt. Food additives have been used in the meat industry for the purposes of flavoring, coloring, for extension of the shelf life, and as antimicrobials (Mehdizadeh *et al*., 2020; Shahbazi *et al*., 2018). Nisin, is a polypeptide produced by certain species of lactic acid bacteria. Nisin was approved as a natural food preservative as it has antimicrobial activities against several species of the microorganisms, particularly against Gram's positive bacteria (Davies *et al*., 1997; Tang *et al*., 2020). Gingerol is the major component of ginger with several reported biological activities. The antimicrobial effects of gingerol were reported against several bacterial species (Park *et al*., 2008; Tang *et al*., 2020). However. the antilisterial activities of nisin, and gingerol still need to be examined.

In the sight of the previous facts, the current study aimed at investigation of the prevalence of *Listeria* spp., particularly, *L. monocytogenes* in the retailed raw buffalo meat and buffalo mince retailed in Egypt. Furthermore, antimicrobial resistance profiling of the identified *L. monocytogenes* isolates was additionally examined using the disk diffusion method. Besides, the antilisterial activities of nisin and gingerol at two concentrations (1% and 2%) were additionally tested.

MATERIALS AND METHODS

Collection of samples

A hundred samples including 50 samples from each of fresh raw buffalo meat (round), and buffalo mince was collected from butchery shops at different sanitation levels in Zagazig city, Egypt. The collected samples were transferred cooled with no delay to the laboratory for bacterial isolation and identification of *Listeria* spp.

Bacteriological examination

Isolation and identification of *Listeria* spp.

Bacterial examination of *Listeria spp.* in

the examined samples was done according to the method of APHA (2001) including the following steps.

Enrichment procedures

Ten grams from each sample were aseptically homogenized in peptone water 1% (90 ml) at 25ºC for 3 minutes at 3000 rpm. The homogenate was then enriched at 37°C for 24 h. Then, one ml of the enriched culture was mixed with 9 ml of Full Fraser broth as a second enrichment procedure and incubated at 37°C for 48 h.

Isolation procedures

A loopful from the enriched culture was streaked onto Oxford agar (Himedia, India) containing *Listeria* Oxford supplement (Himedia, India). Listeria colonies (dew drop-like, black with brown hallow colonies and 1 to 2 mm in diameter) were observed after incubation for 48 h at 35°C. Presumptive colonies of *Listeria* spp. were inoculated into Tryptone Soya broth (TSB) with 0.6% yeast extract as a supplement and kept at 4°C for further identification.

Identification of listeria isolates

Pure and presumptive *Listeria* isolates were identified morphologically, biochemically (FAO/WHO, 2010), and serologically using the Oxoid *Listeria* Test Kit (Oxoid, Basingstoke, Hampshire, England) using the manufacturer's instructions.

Antibiogram of the identified L. monocytogenes

Antimicrobial resistance profiling of the recovered *L. monocytogenes* isolates 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 *L. monocytogenes*. The guidelines of Clinical Laboratory Standards Institute (Clinical and Laboratory Standards Institute, 2021) were applied. The tested strains were evaluated as susceptible, intermediate, and resistant. Multiple Antibiotic Resistance (MAR) index for each strain was determined according to the formula stipulated by Singh *et al.* (2010) as follow:

$$\text{MAR index} = \frac{\text{No. of resistance (Isolates classified as intermediate were considered sensitive for MAR index)}}{\text{Total No. of tested antibiotics}}$$

Experimental trial to test antilisterial effects of nisin, and gingerol against *L. monocytogenes*Preparation of the experimental groups

Food grades of nisin, (SIDLEY chemical, Linyi city, China), and 6-gingerol (Biopurify Phytochemicals, Chengdu, China) at 1.0, and 2.0% concentrations were used in this trial. Buffalo mince-free from *L. monocytogenes* was formulated as meat balls and grouped into five groups. Each group contained five minced meat balls (n = 5 meat pieces/group, meat weight is 100 g). Group 1 was employed as a control and did not receive any treatment, group 2 was treated with nisin 1%, group 3 was treated with nisin 2%, group 4 was treated with gingerol 1%, group 5 was treated with gingerol 2%. Experimental groups were soaked in the food additives for 30 minutes before inoculation with the pathogen. All groups were artificially inoculated with *L. monocytogenes* and incubated for at 35°C for 24 h.

Inoculation of *L. monocytogenes*

The reference strain of *L. monocytogenes* NCTC 13372/ATCC7644 obtained from Animal

Health Institute, Dokki, Giza, Egypt, was used in the experimental trial after refreshment on Oxford agar. The microbial colonies were inoculated into TSB and incubated at 35°C for 24 h. Then, the microbial cells were made as pellets by centrifugation for 15 minutes at 3000 rpm. Each pellet was washed twice in 10 ml of 0.01 phosphate-buffered saline, pH is 7.0, and diluted to $1.0x10^7$ CFU/ ml in PBS for inoculation of the samples. Cell count was determined by serial dilution and subsequent enumeration on Oxford agar (Govaris *et al*., 2010).

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 *L. monocytogenes* counts in the experimental trial were transferred into base-10 logarithms of cfu/g. Data of the experimental trial were analyzed using the one-way ANOVA procedure of SPSS v.23 (SPSS Inc., Chicago, Illinois, USA). Tukey's multiple comparison tests were used to test significant variations among treatment groups. Data were expressed as means ± SD, with a P-value of 0.05 is considered significant.

RESULTS AND DISCUSSIONS

The obtained results of the present study revealed an overall isolation rate of *Listeria spp.* from all examined samples at 34%. The overall isolation rate of *L. monocytogenes* from the examined buffalo samples was 10% (Figure 1). Serological identification of the isolated *Listeria spp.* revealed recovery of six *Listeria* spp. namely, *L. ivanovii, L. welshimeri, L. innocua, L. seeligeri, L. grayi,* and *L. monocytogenes. L. ivanovii* was isolated only from one buffalo mince sample at 2%. *L. welshimeri* was isolated at 8%, and 10% from the examined buffalo meat and buffalo mince, respectively. *L. innocua* and *L. seeligeri* were isolated at 2%, and 4% from buffalo meat, respectively; while isolated at 6%, and 4% from buffalo mince, respectively. *L. grayi* was isolated at 2%, and 6% from the examined buffalo meat and buffalo mince, respectively. *L. monocytogenes* was isolated at 6%, and 14% from the examined buffalo meat and buffalo mince, respectively (Figure 2). In agreement with the recorded results, *L. monocytogenes* was isolated at 4% from each of beef burger, minced meat, and luncheon samples collected from Giza Governorate, Egypt during 2014 (Mohamed *et al*., 2016). *Listeria spp.* was isolated at higher rates (73.9%) from imported frozen beef, and at 43.5% from local beef in Malaysia. In the same study, *L. monocytogenes* was recovered at 75% of the frozen beef samples, and at 30.4% of local meat, but not isolated from buffalo meat (Hassan *et al*., 2001). Besides, *Listeria* spp., and *L. monocytogenes* were isolated from meat sampled in India at 2.4% and 10.2%, respectively (Barbuddhe *et al*., 2002). *Listeria* spp. was additionally isolated from buffalo meat at 29.2% in Iran. The most common *Listeria* spp. recovered in that study was *L. innocua* (75.9%), *L. monocytogenes* (19.1%), *L. welshimeri* (6.4%), *L. seeligeri* (3.5%), and *L. grayi* (1.4%) (Rahimi *et al*., 2012).

Contamination of the buffalo meat and buffalo mince in the present study indicates improper hygienic practices adopted during slaughtering, evisceration, or distribution. *Listeria* spp., and particularly *L. monocytogenes* has the ability to grow in different food matrices such

as meat, dairies, and vegetables and to survive extreme environmental conditions such as high-salt environments and a wide range of temperatures (Khan *et al*., 2016; Shamloo *et al*., 2019). Ingestion of foods contaminated with *L. monocytogenes* might lead to listeriosis, which is a disease that is characterized by severe symptoms including meningitis, encephalitis, abortion, and even death (Castellazzi *et al*., 2018). *L. monocytogenes* was isolated from stool samples collected at Zagazig University hospital, Egypt at 2% (Ahmed *et al*., 2013).

The abuse of antimicrobials during livestock production might lead to the development of drug resistance among several foodborne pathogens. In the current study, the recovered isolates of *L. monocytogenes* showed a 100% resistance to streptomycin. The drug resistance rates for the recovered *L. monocytogenes* isolates in the present study were as following: ampicillin (10%), cephalothin (30%), chloramphenicol (10%), ciprofloxacin (20%), enrofloxacin (30%), erythromycin (70%), gentamicin (80%), kanamycin (40%), nalidixic acid (40%), neomycin (90%), oxacillin (60%), oxytetracycline (50%), and sulfamethoxazole (30%) (Figure 3). Nine of the ten isolated *L. monocytogenes* showed multidrug resistance profiling with an average MAR index of 0.471 (Table 1).

In agreement with the obtained results in the present study, Yucel *et al.* (2005) reported that all *L. monocytogenes* isolates from raw meat products in Turkey were resistant to cephalothin and nalidixic acid, and 66% of isolates were resistant to sulfamethoxazole, ampicillin, and trimethoprim. Similarly, in China, 8.4% of *L. monocytogenes* isolates recovered from retailed food products were resistant to tetracycline, and 1.8% were resistant to ciprofloxacin (Zhang *et al*., 2007). Furthermore, Maćkiw *et al.* (2020) reported that 83% of *L. monocytogenes* isolates were resistant to ampicillin.

An experimental trial was employed to investigate the antilisterial activities of some natural food additives including nisin, and gingerol. The obtained results revealed that both of the used additives did not change the overall acceptability of the buffalo mince in terms of red color, firm in consistency, and fresh odor (Data are not shown). Interestingly, nisin, and gingerol significantly ($P<0.05$) reduced *L. monocytogens* counts, particularly at 2% (Figure 4A). For instances, nisin achieved reduction rates of 11.36%, and 44.84% at 1%, and 2%, respectively; while gingerol achieved reduction rates of 8.44%, and 36.17% at 1%, and 2%, respectively (Figure 4B). In agreement with the obtained results, nisin had significant *in vitro* antilisterial activities (Avery and Buncic, 1997). Furthermore, nisin had significant antibacterial effects against Gram-positive organisms (He *et al.,* 2017). Furthermore, Tang *et al*. (2020) reported clear antimicrobial effects of nisin and gingerol, particularly against spoilage and indicator organisms.

CONCLUSION

The present study demonstrates that buffalo meat and buffalo mince might be considered as potential sources of multidrug-resistant *L. monocytogenes*. Therefore, strict hygienic measures should be adopted during the preparation and processing of meat and meat products before serving to humans. Furthermore, it is highly suggested to use nisin, and gingerol for the control of *L. monocytogenes* in the meat industry.

Table 1. Antimicrobial resistance profiling of the identified *Listeria monocytogenes* isolates using disk diffusion method.

Isolate ID	Resistance profile	MAR index
L. monocytogenes 1	AMP, CN, CH, CP, EN, E, G, K, NA, N, OX, T, S, SXT	1
L. monocytogenes 2	CN, CP, EN, E, G, K, NA, N, OX, T, S, SXT	0.857
L. monocytogenes 3	CN, EN, E, G, K, NA, N, OX, T, S, SXT	0.785
L. monocytogenes 4	E, G, K, NA, N, OX, T, S	0.571
L. monocytogenes 5	E, G, N, OX, T, S	0.428
L. monocytogenes 6	E, G, N, OX, S	0.357
L. monocytogenes 7	E, G, N, S	0.285
L. monocytogenes 8	G, N, S	0.214
L. monocytogenes 9	N, S	0.142
L. monocytogenes 10	S	0.071
Average		0.471

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).

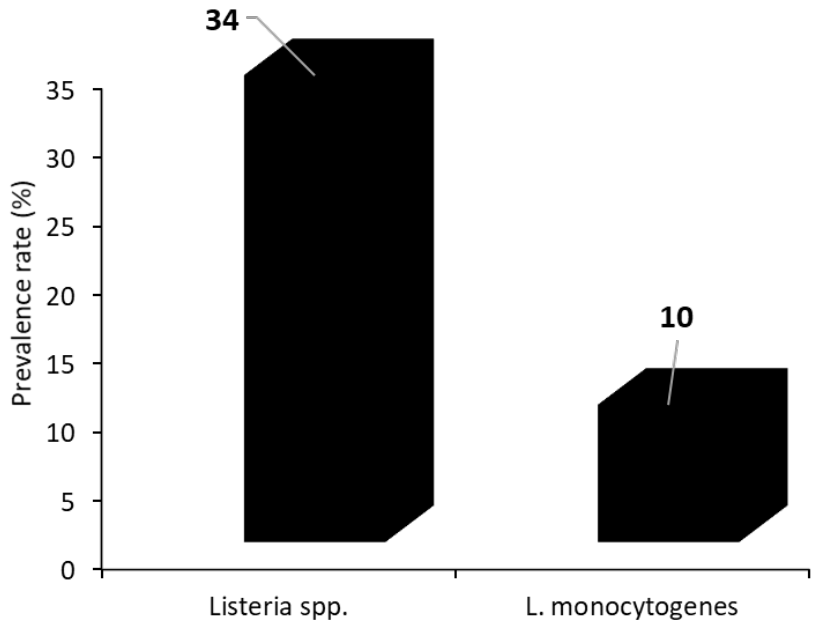

Figure 1. The overall isolation rates of *Listeria* spp., and *L. monocytogenes* from buffalo samples in the present study.

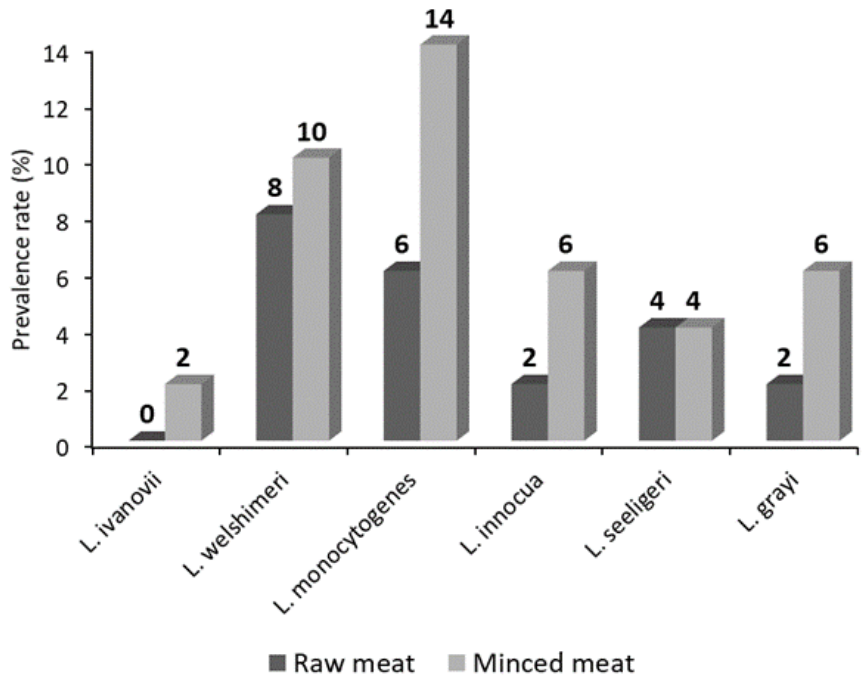

Figure 2. Prevalence rates of different Listeria serotypes recovered from buffalo samples in the present study.

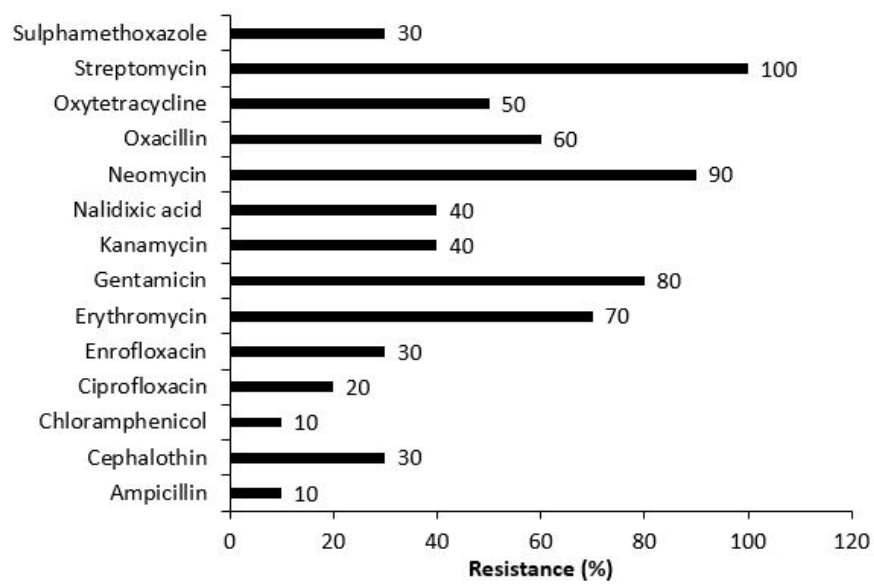

Figure 3. Antibiogram of the recovered *Listeria monocytogenes.*

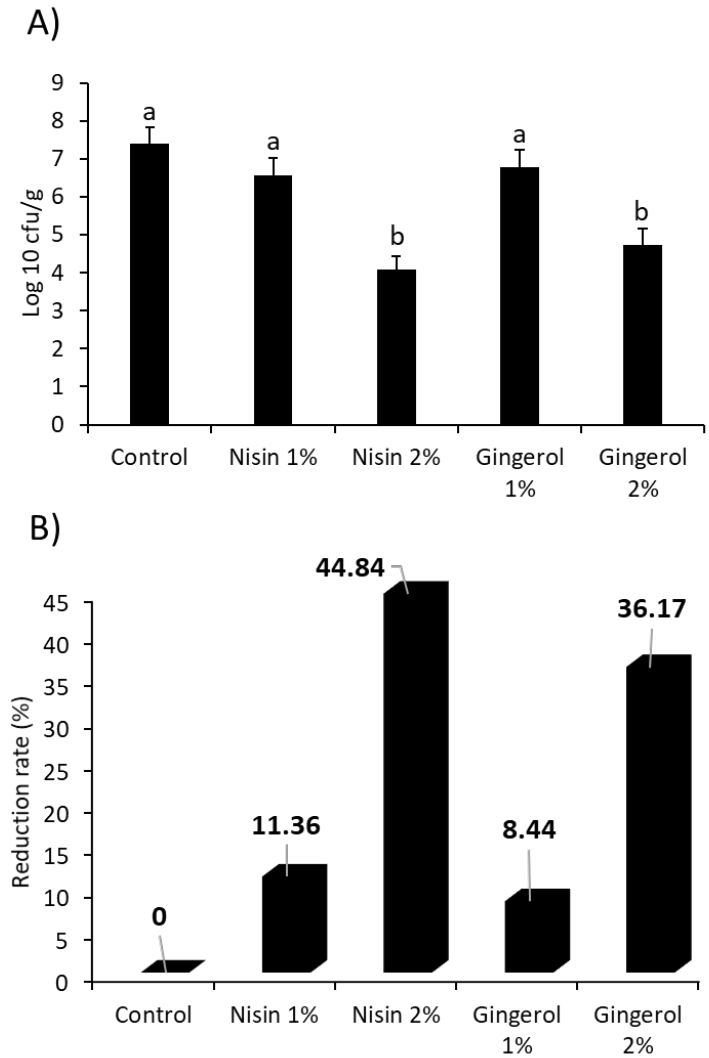

Figure 4. A) Effects of nisin and gingerol on *L. monocytogenes* count (Log 10 cfu/g) in buffalo mince experimentally inoculated with *L. monocytogenes.*

B) The reduction rates (%) of nisin and gingerol against *L. monocytogenes*. Columns with different letter (a, b) are significantly different at $P<0.05$.

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