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A study on the prevalence of multidrug resistant food poisoning *Salmonella* spp. in camel meat and offal with a reduction trial using organic acids

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ABSTRACT

Camel meat is an important source of red meat and essential amino acids in Egypt and other Arab countries. This study aimed at investigation of the prevalence of *Salmonella* spp. in the camel meat and offal (liver, and kidney) retailed in Zagazig city, Egypt. Besides, antibiogram of the recovered *Salmonella* spp. was screened. In addition, a reduction trial for *Salmonella* loads in the prepared camel meat ball using organic acids such as acetic and lactic acids or their combination. The obtained results revealed isolation of *Salmonella* spp., at 20% (12 out of 60 samples). With the highest prevalence in the liver (30%), followed by kidney (20%), and muscle (10%), respectively. *Salmonella* spp., that were isolated were identified serologically as *S.* Enteritidis (33.33%), *S.* Typhimurium (41.66%), *S.* Virchow (8.33%), and *S.* Apeyeme (16.66%). The recovered species showed marked multidrug resistance with the highest resistance against erythromycin, oxacillin, clindamycin, ampicillin, and nalidixic acid. The use of acetic, lactic acids and their combination could significantly reduce *Salmonella* load in the camel meat samples. In conclusion, the use of organic acids, particularly a combination of acetic and lactic acids (1:1, 2%) is of a particular importance in reducing *Salmonella* load in the camel meat.

Introduction

Camel meat, liver, and kidney are considered to be tremendous sources of essential amino acids in a number of global regions. Additionally, it is an excellent source of protein and unsaturated fatty acids, both of which may be essential in mitigating food-related complications (Alao *et al*., 2017). A considerable number of individuals hold the belief that camel meat is similar in composition to that of sheep and cattle, with the exception of lower fat and cholesterol content and comparable protein content (Kadim *et al*., 2008; Darwish *et al*., 2010; El-Ghareeb *et al*., 2019).

Undoubtedly, the majority of microbial contamination that occurs in carcasses occurs throughout the processes of handling and slaughtering, which encompass distribution, evisceration, skinning, and preparation (Morshdy *et al*., 2022). Bacterial contamination of meat is introduced during each stage of processing, starting from slaughter and continuing through transportation and carcass preparation, until it reaches a state suitable for human consumption (Morshdy *et al*., 2018). The microbial burden associated with meat and digestible offal is increased, at least in part, by their elevated protein and moisture content. The microbiological quality of meat is influenced by various factors such as temperature, circulation, and the physiological condition of the animal (Nychas *et al*., 2008). Microbial contamination of camel meat can originate from various sources, including the animal itself (including its skin and excrement), the hands and clothing of the handler, raw ingredients, cleansing water, gathering containers, and equipment (Darwish *et al*., 2018; Morshdy *et al*., 2023). A significant public health concern is the possibility that camel meat could become contaminated with foodborne microorganisms (Ma *et al*. 2023). Severe health complications may result from human infections caused by drug-resistant microorganisms (Alsayeqh *et al*., 2021; Morshdy *et al*., 2021).

*Salmonella*e belong to the family *Enterobacteriaceae*. These coccobacilli are facultative anaerobic, Gram-negative, non-spore-forming organisms that do not ferment lactose. At present, the Kauffmann White Scheme is utilized for the serological identification of over 2500 serotypes. *Salmonella enterica*, which comprises six subdivisions (I, II, IIIa, IIIb, IV, and VI), and *Salmonella* Bongori, which was previously classified as subsp. V, are the two species that comprise the genus *Salmonella* (Brenner *et al*., 2000).

Salmonella is pathogenic to warm-blooded animals and humans, manifesting as gastroenteritis and typhoid, according to the findings of Wray and Wray (2000). Salmonellosis is a prevalent infectious disease in which *Salmonella* bacteria excreted by domesticated animals or contaminated animal products serve as the primary vectors of infection. Illness manifests predominantly within the intestinal tract, where pathogens penetrate the intestinal epithelium and establish an infection. The organisms then proliferate by invading the intestinal cells of the ileum and colon (intracellular parasitism). Upon cell destruction, the organism proliferates and frequently induces inflammation, which ultimately culminates in enteritis. Additionally, extraintestinal manifestations may manifest. *Salmonella* species that manage to breach the intestinal barrier have the potential to propagate throughout the body via the lymphatic and blood vessels. Septicemia may develop if the immune response fails to conquer this infection. Infections affecting specific organs, including bacteraemia, meningitis, septic arthritis, and abortions, may also ensue. According to Sams (2001), non-typhoidal salmonellosis (gastrointestinal tract infection) in humans can vary in severity from moderate to severe. It is classified as a self-limiting infection that affects the lower intestinal tract. The range of infectious doses was 10,000 to 1,000,000 cells.

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Symptoms manifest between 12 and 36 hours following the ingestion of contaminated food. These symptoms comprise nausea, vomiting, severe diarrhea, fever, abdominal cramping, and malaise.

Organic acids, including citric, acetic, and lactic acids, are widely acknowledged for their safety profile and are widely employed in the meat and poultry sectors to mitigate bacterial contamination on the surfaces of carcasses. It has been determined that these interventions reduce foodborne pathogens such as *Salmonella* Typhimurium, E. coli O157:H7, and Listeria monocytogenes that are commonly found on contaminated surfaces of meat or poultry (Fabrizio *et al*., 2002).

Hence, the objective of this research endeavor was to assess the prevalence of different *Salmonella* species in the camel meat and offal retailed in Zagazig city, Egypt. The antibiogram and virulence attributes of the identified *Salmonella* spp. were also examined. An experiment was conducted to determine whether diluted acetic acid, diluted lactic acid, or an acid cocktail containing equal volumes of acetic acid and lactic acid could be used to reduce *Salmonella* Typhimurium load on the prepared camel meat balls.

Materials and methods

Collection of samples

Sixty samples of muscle, liver, and kidney were obtained from camels that were selected at random. Samples were obtained promptly subsequent to the slaughter process from butcher shops located in Zagazig city, Egypt. During antimortem inspection, animals were checked, and each animal was robust, active, and free from any diseases. The specimens were promptly evaluated after being transported to the laboratory in aseptic conditions in sterile plastic containers. The isolation of *Salmonella* spp. was accomplished by bacteriological analysis of the collected samples.

Sample preparation

To obtain a homogenate of 1/10 dilutions, 25 g of each camel meat sample was homogenized in 225 mL of sterilized buffered peptone water (BPW) (0.1%) (Oxoid CM0509, UK) for a duration of 2 minutes at 2500 revolutions per minute. The produced samples subjected to incubation at 37°C for duration of 24 hours.

Isolation and identification of Salmonella spp.

The recovery and isolation of *Salmonella* spp. followed the methodology outlined in ISO 6579-1 (2017). To summarize, one mL of the incubated BPW was transferred to nine mL of sterile, chilled Rappaport Vassiliadis (RV) broth (Oxoid CM0669, UK) followed by incubation at 42°C for 24 hours. A loopful was then taken from each enriched sample and incubated at 37°C for 24 hours on Xylose-Lysine Desoxycholate (XLD) agar (Oxoid CM0469, UK). For further analysis, five pure colonies (pink colonies with or without a black center) were re-purified in the same medium (XLD) and preserved at -20°C in glycerol for further biochemical and serological identification.

Biochemical identification

A re-inoculation of all preserved colonies was performed by adding them to tryptic soy broth (TSB; Oxoid, Basingstoke, UK) and incubating it at 37°C for 24 hours. After streaking a loopful of the turbid incubated broth onto XLD, it was incubated at 37°C for 24 hours. The isolates that were retrieved were subjected to biochemical tests as delineated by Kreig and Holt (1984).

Serological identification

Biochemical isolates that had been confirmed underwent serological analysis in accordance with Kauffman's (1974) methodology using polyvalent and monovalent O and H antisera (Denka-Seiken, Tokyo, Japan).

Molecular confirmation

By simmering at 100°C for 15 to 20 minutes, bacterial DNA was extracted according to Elafify *et al*. (2019). The DNA was stored at -20°C until use. In order to validate the acquired *Salmonella* isolates, PCR was employed to analyze invasion A (*inv*A), a molecular biomarker specific to *Salmonella* spp. A 244-bp PCR product was generated for *inv*A amplification using two oligonucleotide primers (F: 5'-GTGAAATTATCGCCACGTTCGGGCAA-3'; R:5'TCATCGCACCGTCAAAGGAACC-3') (Shanmugasamy *et al*., 2011). The procedure for PCR amplification was outlined by Elafify *et al*. (2022).

Testing for susceptibility to antimicrobials

Phenotypic analysis was performed on the *Salmonella* isolates that were recovered in order to profile their antimicrobial resistance. Amikacillin (OX) (1 µg), ampicillin (AM) (10 µg), clindamycin (CL) (10 µg), cefotaxime (CF) (30 µg), cefazolin (CZ) (30 µg), ciprofloxacin (CP) (5 µg), erythromycin (E) (15 µg), gentamicin (G) (10 µg), kanamycin (K) (30 µg), nalidixic acid (NA) (30 µg), were the antimicrobials that were tested. The procedure outlined by Mary and Usha (2013) was adhered to. To summarize, the reintroduced isolates were applied onto the Muller-Hinton agar surface, and the antimicrobial discs were positioned above the agar medium with gentle pressure applied with sterile forceps. Each plate was incubated at 37°C for 24 hours. The inhibition zone's diameter was quantified and compared to the standards in accordance with the CLSI (2015) guidelines. The calculation of the Multiple Antibiotic Resistance (MAR) index for each *Salmonella* isolate was performed using the equation outlined by Singh *et al*. (2010):
MAR index= Number of resistant isolates divided by Total number of antibiotics tested.

Experimental investigation utilizing natural additives to inactivate Salmonella

The process of bacterial preparation

As standard strains, four confirmed *S.* Typhimurium recovered in the present investigation was utilized. To allow for rejuvenation, a loopful was extracted from each glycerol stock isolate separately, inoculated into TSB (Oxoid, Basingstoke, UK), and subsequently subjected to 18 hours of incubation at 37°C. After selecting a single pure colony, it was distributed onto XLD and incubated at 37°C for 18 hours. An individual purified isolate was introduced into TSB and subjected to incubation at 37°C for duration of 18 hours in order to achieve a final concentration of 10^9 CFU/mL. The cocktail pathogens were inoculated into one kilogram of a well-prepared camel mince to achieve an approximate concentration of 10^6 CFU/mL (Elafify *et al*., 2019).

A reduction trial for *S.* Typhimurium in the prepared camel minces using organic acids

For the purpose of reducing the *S.* Typhimurium burden of the prepared and inoculated camel mince, diluted acids were utilized and 30 camel meat balls (33 g for each meat ball) that inoculated with the tested *Salmonella* strains were prepared. The effect of different concentrations of acetic acid (1% and 2%), lactic acid (1% and 2%), and an acid cocktail (equal parts acetic acid and lactic acid 1:1 (2%)) on the total *S.* Ty-

phimurium count was investigated using six experimental groups. Each experimental group contained 5 meat balls; the first group left with no treatment; the second group was immersed in acetic acid 1%; the third group was immersed in acetic acid 2%; the fourth group was immersed in lactic acid acid 1%; the fifth group was immersed in lactic acid 2%; the six group was immersed in the acid cocktail containing 1:1 of 2% acetic and lactic acids. The microbiological analysis was performed as previously stated. The reduction percentage was calculated as previously reported (Darwish *et al*., 2017).

Statistical analysis

For reduction investigations, Dunnett's test was employed to compare measurements with those of the control group (0% acid) (Gomez and Gomez, 1984). P< 0.05 was utilized to establish statistical significance in all analyses through the utilization of the JMP statistical application (SAS Institute Inc., Cary, NC).

Results and Discussion

Salmonella, a notable foodborne pathogen, poses a significant public health concern due to its involvement in an estimated 1.3 billion instances of food poisoning and 155,500 fatalities annually on a global scale (Sun *et al*., 2021). Annually, approximately 1.35 million infections, 26,500 hospitalizations, and 420 fatalities are attributed to non-typhoidal *Salmonella* in the United States. These events incur an estimated $400 million in medical expenses (CDC, 2019). Salmonellosis ranked second in the European Union in terms of reported cases of foodborne illness in 2021, trailing only campylobacteriosis. The 60,050 cases represented a 14.3% surge in the EU notification rate when compared to the previous year, 2020 (EFSA, ECDC, 2021). Furthermore, it has been reported that *Salmonella* caused approximately 70% to 80% of infectious illness outbreaks in China (Sun *et al*., 2021). Despite being a prominent foodborne zoonotic disease in Egypt, the country currently lacks comprehensive national surveillance and dependable statistical data on its prevalence and socioeconomic impact (Elshebrawy *et al*., 2021). In the current study, *Salmonella* spp., was isolated at 20% (12 out of 60 samples). With the highest prevalence in the liver (30%), followed by kidney (20%), and muscle (10%), respectively (Fig. 1, Table 1). *Salmonella* spp., that were isolated were identified serologically as *S.* Enteritidis (33.33%), *S.* Typhimurium (41.66%), *S.* Virchow (8.33%), and *S.* Apeyeme (16.66%), which correspond to four distinct serotypes (Fig. 2). Comparable serotypes were detected and isolated in slaughtered camels in Egypt. Sallam *et al.* (2024) reported that *S.* Enteritidis, *S.* Typhimurium, S. Cerro, and S. Montevideo were the most prevalent serovars with incidences of 25 % (32/128), 15.6 % (20/128), 15.6 % (20/128), and 12.5 % (16/128), respectively. According to molecular identification, every isolate contained the *inv*A gene (Fig. 3). *Salmonella* spp. utilized the latter as an invasion gene and biomarker, which facilitates bacterial invasion of host cells and is responsible for the development of pathogenesis (Rahn *et al*., 1992). *Salmonella* spp., isolated from camel meat most likely contained the *inv*A gene as well (Sallam *et al*., 2024).

The absence of adequate veterinary oversight during intensive livestock production and the unregulated application of antimicrobials contributed to the emergence of multidrug-resistant contaminated pathogens (Abd-Elghany *et al*., 2015). Notably, multidrug resistance was observed in all *Salmonella* isolates examined in the current investigation, with the highest levels of resistance (100%) to erythromycin, oxacillin, clindamycin, ampicillin, and nalidixic acid (Fig. 4, Table 2). A number of studies have documented the isolation of *Salmonella* spp., that are resistant to multiple drugs, including erythromycin, oxytetracycline, and cefotaxime, from food subjects (Miranda *et al*., 2009; Doosti *et al*., 2017; Sallam *et al*., 2024).

To reduce *Salmonella* load, an experiment was conducted utilizing diluted acids such as acetic, lactic, and acid cocktail. After immersion of the prepared camel meat balls in acetic or lactic acids, *S.* Typhimurium count was significantly (p< 0.05) reduced to 94.98% and 83.33% after

Table 1. Sources and serotypes of *Salmonella* spp. isolated from the examined samples

Pathotypes	Serotypes	Distribution of *Salmonella* positive isolates			
		Camel meat	Camel liver	Camel kidney	Total number
S. Enteritidis	O1.9.12:Hg,m	1	2	1	4
S. Typhimurium	O1.4.5.12:H:1.2	1	2	2	5
S. Virchow	O6.7.14:H:r:1.2	0	1	0	1
S. Apeyeme	O8,20 H:Z38:	0	1	1	2
Total		2	6	4	12

Table 2. Virulence and antimicrobial characterization of *Salmonella* spp. isolates recovered from the examined samples

No.	Type of sample	*Salmonella* spp	Antimicrobial resistance profile	MAR index	Biomarker and virulence gene (*InvA* gene)
1	Liver	*S.* Enteritidis	E, OX, NA, CL, AM, K, CF, CP, T, AK, SXT, CZ, G, IPM	1	+
2	Liver	*S.* Enteritidis	E, OX, NA, CL, AM, K, CF, CP, T, AK, SXT, CZ	0.86	+
3	Kidney	*S.* Enteritidis	E, OX, NA, CL, AM, K, CF, CP, T, AK, SXT	0.79	+
4	Meat	*S.* Enteritidis	E, OX, NA, CL, AM, K, CF, CP, T, AK	0.71	+
5	Liver	*S.* Typhimurium	E, OX, NA, CL, AM, K, CF, CP, T, AK, SXT, CZ, G, IPM	1	+
6	Liver	*S.* Typhimurium	E, OX, NA, CL, AM, K, CF, CP, T, AK, SXT, CZ, IPM	0.93	+
7	Kidney	*S.* Typhimurium	E, OX, NA, CL, AM, K, CF, CP, T, AK, SXT,CZ,G	0.93	+
8	Kidney	*S.* Typhimurium	E, OX, NA, CL, AM, K, CF, CP	0.57	+
9	Meat	*S.* Typhimurium	E, OX, NA, CL, AM	0.36	+
10	Liver	*S.* Virchow	E, OX, NA, CL, AM, K, CF, CP, T, AK, SXT	0.79	+
11	Liver	*S.* Apeyeme	E, OX, NA, CL, AM, K, CF, CP, T, AK, SXT	0.79	+
12	Kidney	*S.* Apeyeme	E, OX, NA, CL, AM, K, CF, CP, T	0.64	+

CL: Clindamycin; K: Kanamycin; NA: Nalidixic acid ; CF: Cefotaxime ; SXT: Sulphamethoxazol; CZ: Cefazolin; T: tetracycline; E: Erythromycin ; AM: Ampicillin ; G: Gentamicin; IMP: Imipenem ; AK: Amikacin; CP: Ciprofloxacin; OX: Oxacillin

imm l 2%

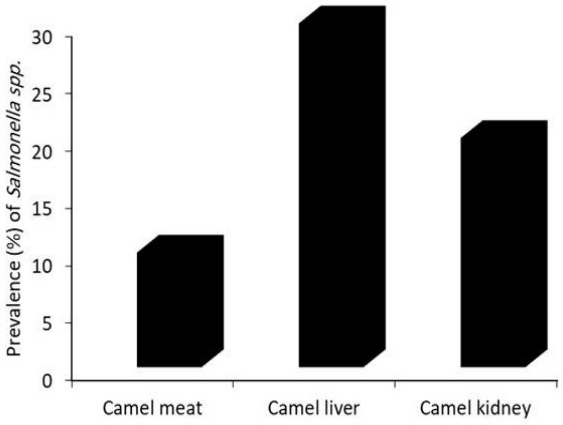

Fig. 1. Prevalence (%) of *Salmonella* spp., in the examined camel meat, liver and kidney.

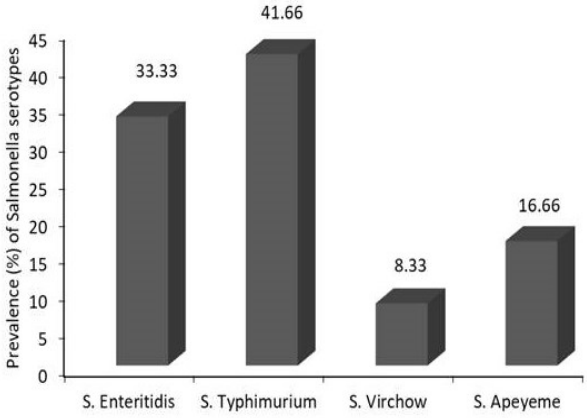

Fig. 2. Prevalence (%) of different *Salmonella* Serovars in the examined camel samples.

Fig. 3. Detection of *inv*A gene in the recovered *Salmonella* spp. isolates using PCR.

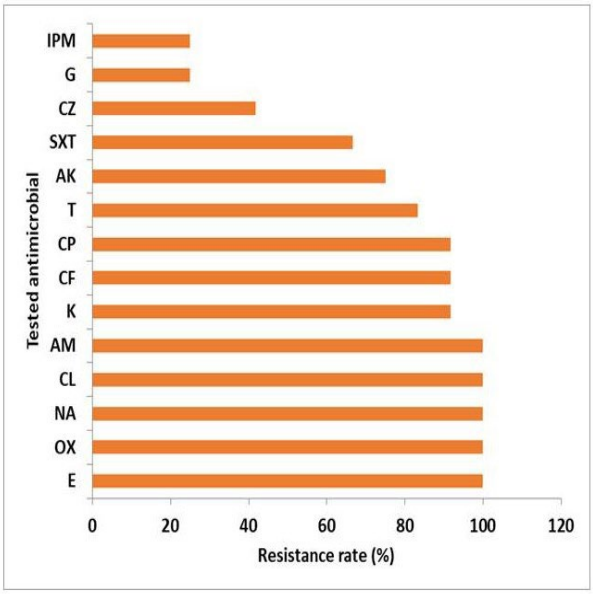

Fig. 4. Antimicrobial resistance rates (%) among the recovered *Salmonella* from the examined camel samples.

reduced *S.* Typhimurium count to 95.54% and 85.77%, respectively. The prepared acid cocktail 2% achieved the most significant reduction in *S.* Typhimurium count to 78.32% (Fig. 5). It is noteworthy to mention that the acid concentrations utilized in the analysis did not have an impact on the sensory attributes of the camel meat samples. Consistent with this finding, Menconi *et al.* (2013) assessed the efficacy of various organic acid (OA) wash solution combinations (acetic, citric, and propionic acid) in inhibiting the growth of spoilage bacteria and pathogens on raw chicken skin while refrigerated for storage. When skin samples were treated with the OA wash solution, significant reduction ($p < 0.05$) was observed, and spoilage organisms were not recovered at any time point. According to these findings, 0.2 to 0.8% concentrations of an equal-percentage mixture of this OA combination may enhance the food safety properties of raw poultry by reducing the presence of pathogens and spoilage organisms.

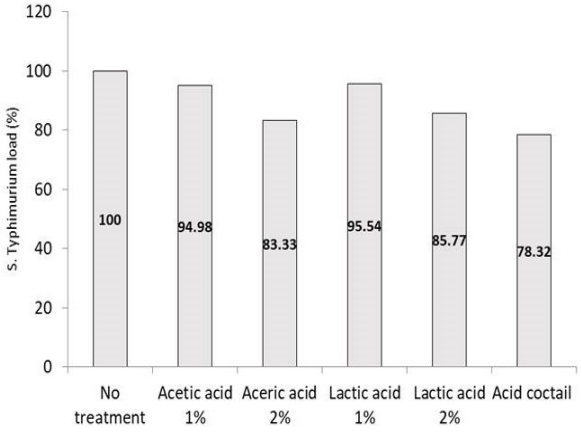

Fig. 5. The effect of acetic, lactic acids and their combination on *S.* Typhimurium load in the prepared camel meat balls.

A plausible rationale for the diminished microbial burden observed in the camel samples when diluted acid solutions were utilized is that such solutions might decrease the pH value of the meat products, thereby creating an unfavorable environment for bacterial growth and multiplication. Consistent with this assumption, Koutsoumanis *et al.* (2006) observed a noteworthy correlation between the pH of meat and the rate of growth of *Enterobacteriaceae*, and pseudomonads. In agreement with the present findings, camel meat dipped in solutions containing potassium sorbate (1.5% w/w), sodium acetate (10% w/w), sodium lactate (5% v/v of 60% solution), or trisodium citrate (1.5% w/w), either alone or in combination with Bifidobacterium breve cell suspension could show significant inhibition of the spoilage organisms (Al-Sheddy *et al.*, 1999).

Conclusion

The current study revealed isolation of *Salmonella* spp., from camel meat, liver, and kidney at 20%. Four *Salmonella* spp. were recovered, namely *S.* Enteritidis, *S.* Typhimurium, *S.* Virchow, and *S.* Apeyeme. The recovered isolates showed marked multidrug resistance profiling. The use or organic acids such as acetic and lactic acids or their combination are of value in reducing *Salmonella* spp. load in the camel meat.

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Conflict of interest

The authors declare that they have no conflict of interest.

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