



Occurrence of Staphylococcus Aureus, Campylobacter Jejuni, Listeria Monocytogenes, and Arcobacter Butzleri in Poultry Meat

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ARTICLE INFO

Keywords:

Arcobacter butzleri, *Listeria monocytogenes*, *Campylobacter jejuni*, *Staphylococcus aureus*, Poultry meat, Occurrence

Received: Jun, 27, 2025

Accepted: Aug, 12, 2025

Published: Dec, 25, 2025

ABSTRACT

Arcobacter butzleri, *Listeria monocytogenes*, *Staphylococcus aureus*, *Campylobacter jejuni* are significant foodborne pathogens regarding the consumption of raw poultry meat. An existing survey was conducted to assess the occurrence of *S. aureus*, *C. jejuni*, *A. butzleri* and *L. monocytogenes* in raw poultry meat samples. Ninety-four raw ostrich, turkey, chicken and quail meat samples were collected and subjected to culture-based analysis. *S. aureus*, *C. jejuni*, *A. butzleri* and *L. monocytogenes* isolates were confirmed by standard biochemical techniques. Occurrence of *A. butzleri*, *C. jejuni*, *L. monocytogenes* and *S. aureus* in poultry meat samples was 11.45%, 17.70%, 1.04% and 16.66%, respectively. *L. monocytogenes* was absent in chicken, turkey and ostrich meat samples. Only one quail meat (4.16%) was positive for *L. monocytogenes*. The uppermost contamination rate with *A. butzleri*, *C. jejuni* and *S. aureus* was found in chicken (25%), turkey (25%) and turkey (25%) meat samples, respectively. Concurrent occurrence of *A. butzleri* + *C. jejuni* + *S. aureus* bacteria amid the examined poultry meat samples was 2.08%. This is an initial report of simultaneous isolation of *A. butzleri*, *C. jejuni*, *L. monocytogenes* and *S. aureus* in poultry meat samples. Adequate cooking of poultry meat can diminish the risk of foodborne diseases owing to *A. butzleri*, *C. jejuni*, *L. monocytogenes* and *S. aureus* bacteria.

1. INTRODUCTION

Poultry meat is an excellent source of numerous vitamins and minerals [1]. Human involvement in the poultry meat inspection and purchase augmented the probabilities of the occurrence of bacterial contamination and complicated foodborne diseases [1-8]. *Staphylococcus aureus* (*S. aureus*) is a Gram-positive, catalase positive, and cocci-shaped bacterium characteristically originate from the respiratory tract and on the skin.

S. aureus is responsible for unadorned nosocomial and community-acquired infections, foodborne diseases [9-13]. Occurrence of numerous kinds of gastrointestinal disorders recognized by abdominal cramps, nausea, vomiting, weakness and diarrhea are accredited to *S. aureus*. *Listeria monocytogenes* (*L. monocytogenes*) is a Gram-positive, rod-shaped and opportunistic bacterium which is extensively banquet in the environment.

The bacterium can cause severe diseases including meningoencephalitis, septicemia, mastitis, metritis, keratoconjunctivitis, iritis and abortion in humans and animals, principally distressing newborn, pregnant, and immunocompromised persons [14,15]. It is also responsible for occurrence of foodborne diseases recognized by fever and in some cases fetal abortion in both human and animal [14, 15]. *Campylobacter* species are Gram-negative and microaerophile bacteria considered as the most routine reason of gastroenteritis. *Campylobacter jejuni* (*C. jejuni*) is the most significant bacterium of this genus accompanying with human diseases [16, 17]. Clinical manifestations of campylobacteriosis are comprise diarrhea, abdominal cramp, and fever [16]. Bloody diarrhea may present in complicated cases. The bacterium is also responsible for occurrence of Guillain Barré Syndrome recognized with immune-mediated neuropathies, ataxia, areflexia, and ophthalmoplegia [17]. *Arcobacter* species are Gram-negative spiral-shaped bacteria of the *Campylobacteraceae* family. *Arcobacter butzleri* (*A. butzleri*) is one of the most important species in the genus *Arcobacter*. *A. butzleri* has developed as a significant foodborne zoonotic bacterium responsible for occurrence of diverse clinical syndrome including mastitis, diarrhea, and abortion in animals and diarrhea, peritonitis, endocarditis and gastroenteritis in humans [18,19]. Poultry meat and its products are measured as reservoirs of *S. aureus*, *C. jejuni*, *A. butzleri* and *L. monocytogenes* [20, 21]. Poultries and infected staffs may act as transporters of *S. aureus*, *C. jejuni*, *A. butzleri* and *L. monocytogenes* [20, 21]. Thus, it is indispensable to assess the presence of these pathogenic foodborne bacteria in poultry meat. At present, there is inadequate data rendering the occurrence of *S. aureus*, *C. jejuni*, *A. butzleri* and *L. monocytogenes* in poultry meat in Iran. Thus, a current survey was done to measure the occurrence of *S. aureus*, *C. jejuni*, *A. butzleri* and *L. monocytogenes* in ostrich, turkey, chicken, and quail meat samples collected from Isfahan, Iran.

2. MATERIALS AND METHODS

2.1. Samples

From May to August 2019, 94 raw poultry meat samples including ostrich (n= 24), turkey (n=24), chicken (n= 24), and quail (n= 24) were arbitrarily collected from the retail centers of Isfahan, Iran.

Sampling was done from the femur muscle of poultries. Samples (40 g, in sterile glass bottles) were rapidly transferred to laboratory at 4 °C.

Isolation and identification of *S. aureus*

Isolation was performed from the 25 g of samples. Isolation criteria was done according to method labelled beforehand [22]. Samples were primarily homogenized with 225 mL buffered peptone water (Merck, Germany) using Stomacher (Interscience, Saint-Nom, France). Afterward, samples (5 mL) were added to Trypticase Soy Broth (50 mL) (Merck, Germany) contained NaCl (10%) and sodium pyruvate (1%). Incubation was done at 35 °C for 18 h. Afterward, a loopful of culture was transferred into Baird-Parker agar (Merck, Germany) contained egg yolk tellurite emulsion. Incubation was done for 24 h at 37 °C. *S. aureus* identification was performed by biochemical tests [22].

Isolation and identification of *L. monocytogenes*

A similar volume of samples were primarily homogenized with 225 mL Listeria enrichment broth (Merck, Germany). Incubation was done for 24 h at 37 °C. Afterward, a milliliter of samples was transferred to Frazer broth (9 mL) (Merck, Germany). Incubation was done for 24 h at 37 °C. Palcam agar (Merck, Germany) and Oxford agar (Merck, Germany) media were used for other enrichment. Incubation was done for 48 h at 35 °C. Three black colonies with black sunken were cultured on Tryptone Soy agar (Merck, Germany) contained yeast extract (0.6%). Incubation was done for 24 h at 37 °C. *L. monocytogenes* was identified by biochemical tests [23].

Isolation and identification of *C. jejuni*

A similar volume of samples were primarily homogenized with 225 mL of Preston enrichment broth (HiMedia, India) contained sheep blood (5%) and *Campylobacter* selective supplement IV (HiMedia, India). Microaerophilic incubation was done for 24 h at 42 °C. Afterward, the enrichment (0.1 mL) was added to *Campylobacter* selective agar (HiMedia, India) contained specific antibiotic (HiMedia, India) and sheep blood (5%). Microaerophilic incubation was done for 48 h at 42 °C. *C. jejuni* was identified by biochemical tests [24].

Isolation and identification of *A. butzleri*

A similar volume of samples were primarily homogenized with 225 mL of Arcobacter broth contained trimethoprim (64 mg/l), amphotericin B, novobiocin (32 mg/l), cefoperazone, 5-fluorouracil (100 mg/l) and teicoplanin (Oxoid, UK). Incubation was done for 48 h at 30 °C. Afterward, bacteria were transferred to Arcobacter agar (Oxoid, UK) contained the same ingredients as above and also sheep blood. Incubation was done for 48 h at 30 °C. *A. butzleri* was identified by biochemical tests [25].

2.2. Numerical examination

Numerical examination was performed using the SPSS 21.0 (Chicago, USA). Chi-square and Fisher's exact two-tailed tests were performed for numerical analysis of data. *P* value <0.05 was

Table 1. Occurrence of foodborne bacteria in numerous kinds of poultry meat samples.

Types of meat samples	N samples collected	N samples positive for bacteria (%)			
		<i>A. butzleri</i>	<i>C. jejuni</i>	<i>L. monocytogenes</i>	<i>S. aureus</i>
Chicken	24	6 (25)	8 (33.33)	-	4 (16.66)
Turkey	24	3 (12.50)	6 (25)	-	6 (25)
Ostrich	24	1 (4.16)	1 (4.16)	-	3 (12.50)
Quail	24	1 (4.16)	2 (8.33)	1 (4.16)	3 (12.50)
Total	96	11 (11.45)	17 (17.70)	1 (1.04)	16 (16.66)

Table 2 signifies the combined occurrence of foodborne bacteria amid the poultry meat samples. Total occurrence of *A. butzleri* + *C. jejuni*, *A. butzleri* + *S. aureus*, *C. jejuni* + *L. monocytogenes*, *C. jejuni* + *S. aureus*, *L. monocytogenes* + *S. aureus* and *A. butzleri* + *C. jejuni* + *S. aureus* bacteria amid the

determined as numerically significant level [26-32].

3. RESULTS

Table 1 signifies the occurrence of foodborne bacteria in studied poultry meat samples. Occurrence of *A. butzleri*, *C. jejuni*, *L. monocytogenes* and *S. aureus* in examined poultry meat samples was 11.45%, 17.70%, 1.04% and 16.66%, respectively. Used technique failed to detect any *L. monocytogenes* bacteria amid the ostrich, turkey, and chicken meat samples. Additionally, only one quail sample (4.16%) was positive for *L. monocytogenes*. Statistically significant difference was obtained amid type of poultry meat samples and occurrence of bacteria (*P* <0.05).

examined poultry meat samples was 4.16%, 4.16%, 1.04%, 4.16%, 1.04% and 2.08%, respectively. Applied method failed to detect *A. butzleri* + *L. monocytogenes* bacteria in all examined poultry meat samples.

Table 2. Combined occurrence of foodborne bacteria amid the poultry meat samples.

Types of meat samples (N. samples)	N samples positive for bacteria (%)						
	<i>A. butzleri</i> + <i>C. jejuni</i>	<i>A. butzleri</i> + <i>L. monocytogenes</i>	<i>A. butzleri</i> + <i>S. aureus</i>	<i>C. jejuni</i> + <i>L. monocytogenes</i>	<i>C. jejuni</i> + <i>S. aureus</i>	<i>L. monocytogenes</i> + <i>S. aureus</i>	<i>A. butzleri</i> + <i>C. jejuni</i> + <i>S. aureus</i>
Chicken (24)	3 (12.50)	-	2 (8.33)	-	1 (4.16)	-	1 (4.16)
Turkey (24)	1 (4.16)	-	1 (4.16)	-	2 (8.33)	-	1 (4.16)
Ostrich (24)	-	-	1 (4.16)	-	1 (4.16)	-	-
Quail (24)	-	-	-	1 (4.16)	-	1 (4.16)	-
Total (96)	4 (4.16)	-	4 (4.16)	1 (1.04)	4 (4.16)	1 (1.04)	2 (2.08)

4. DISCUSSION

Yearly, an assessed 75 million diseases, 300,000 hospitalizations, and 6,000 deaths are caused by foodborne diseases in the United States [33]. Amid these cases, 31 recognized pathogens cause 9.5 million diseases, 55,000 hospitalizations, and 1200 deaths [34]. The current investigation showed that

the whole occurrence of *S. aureus*, *C. jejuni*, *A. butzleri* and *L. monocytogenes* bacteria amid the examined raw poultry meat samples were 16.66%, 17.70%, 11.45% and 1.04%, respectively. High occurrence of bacteria in examined poultry meat samples is may be due to the primary presence of bacteria in studied poultries, occurrence of cross

contamination through diverse stages of the slaughter, transmission of bacteria from infected staffs and workers to poultry carcasses and finally, transmission of bacteria from contaminated water used from washing of poultry carcasses. Subordinate occurrence of *S. aureus* was conveyed in food stuffs from Italy [35] and Netherlands [36]. Occurrence of *S. aureus* in meat in Brazil [37], Egypt [38], and Denmark [39] were 21.72%, 40.80%, 71.50% and 52.00%, respectively. Moreover, role of poultry meat as a reservoir of *S. aureus* bacteria was also recognized in samples collected from Australia [40], United Kingdom [41] and United States [42]. Our findings unveiled that turkey and chicken meat samples may be the significant reservoirs of *S. aureus* bacteria.

Poultry act as an imperative reservoir of *A. butzleri* [43, 44]. It is considered as a source of infection transmission and spread [43-45]. Poultry intestines have been projected to harbor *Arcobacter* and contaminate the slaughterhouses throughout carcasses processing thereby pretty the chances of additional contamination. Occurrence of *Arcobacter* in chicken meat samples collected from Nigeria [46], Malaysia [47], Korea [48], and Spain [43] was 37.50%, 32.00%, 39.20%, 0-100%, 23.20% and 64.30%, respectively. Rahimi (2014) [44] conveyed that the occurrence of *Arcobacter* strains amid raw chicken, turkey, quail, partridge, duck, ostrich and geese meat samples were 28.00%, 11.00%, 12.00%, 7.50%, 11.40%, 3.30 and 8.00%, respectively. He revealed that 90.10% of *Arcobacter* isolates were related to *A. butzleri*. de Oliveira et al (2018) [49] conveyed that the occurrence of *Arcobacter* spp. in chicken meat samples was 18.30% in which 63.60% of isolates were related to *A. butzleri*. Molva and Atabay (2016) [50] conveyed that the occurrence of *Arcobacter* species amid the chicken meat samples was 55% in which 80% of isolates were *A. butzleri*. *L. monocytogenes* has lower occurrence in comparison with other tested bacteria. Perković et al. (2016) [51] specified that the occurrence of *L. monocytogenes* amid the poultry meat samples was 1%. They signified that turkey meat was the most commonly contaminated (2%). Elmali et al. (2015) [52] described that the occurrence of *Listeria* species in chicken meat samples were 47.50% in which 94.70% of the isolates were identified as *L. monocytogenes*. Osaili et al. (2011) [53] specified

that *L. monocytogenes* was recovered from 9.40% raw broiler samples which was higher than our findings. Meyer et al. (2012) [54] signified that the *Listeria* species were isolated from 6% of poultry meat product samples which was also higher than our findings. In keeping with this, various occurrence rates of *L. monocytogenes* has been conveyed in diverse parts of the world ranging from 8% to 99% [53,55,56]. Schäfer et al. (2018) [57] described the contamination rate of raw chicken meat samples 44.19%. *C. jejuni* is well adapted to growth and survival in poultry meat and dependent products. Mezher et al. (2016) [58] conveyed that the occurrence of *Campylobacter* species in chicken meat samples were 6.80% in which 27.27% of isolates were identified as *C. jejuni*. Whole occurrence of *C. jejuni* amid the poultry meat samples collected from Iraq [59], Iran [60], Pakistan [61], and India [62] was 10%, 6.84%, 40%, and 26.30%, respectively. Boost occurrence of *Campylobacter* species was also conveyed in poultry meat samples collected from European countries (29-41%) [63, 64]. As observed, diverse researches conveyed various occurrence rates of bacteria. The variance in data proposes that season, time and place of sampling, method of sampling, types of samples and even laboratory methods applied in researches may affect the outcomes of occurrence rate. Moreover, Difference hygienic levels of poultry flocks and shopping centers may affect the occurrence of bacteria in diverse investigations. Microbiology has been developed in food sciences in recent years [65-74].

5. CONCLUSION

Put together, an existing survey was conducted to assess the occurrence of *S. aureus*, *C. jejuni*, *A. butzleri* and *L. monocytogenes* foodborne bacteria in raw ostrich turkey, chicken, and quail meat samples. Furthermore, chicken and turkey had the higher contamination rate. Some samples were simultaneously contaminated with two or three foodborne bacteria which demonstrate the boost importance of poultry meat as a reservoir of pathogenic bacteria. An existing survey is an initial report of simultaneous presence of *A. butzleri*, *C. jejuni*, *L. monocytogenes* and *S. aureus* in ostrich, turkey, chicken, and quail meat samples. Our findings revealed that only quail meat was reservoir for *L. monocytogenes*. Appropriate time and adequate temperature through the cooking of

poultry meat samples can reduce the risk of transmission of *A. butzleri*, *C. jejuni*, *L. monocytogenes* and *S. aureus* bacteria to human. Nevertheless, supplementary surveys can determine additional information regarding the risk of *A. butzleri*, *C. jejuni*, *L. monocytogenes* and *S. aureus* bacteria in poultry meat.

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