

Original Research Article

The Prevalence and Distribution of Enteric Bacteria in Meat and Meat Products

Abstract

Food-borne diseases are the most serious international health issue, causing economic losses and lives. The enteric bacteria are the most difficult bacterial contamination of raw and processed beef products worldwide. It is also the most prevalent type of food poisoning. **Aim:** The current study sought to determine the presence and distribution of enteric bacteria associated with various types of meat (red and white) and their fresh and frozen products. **Methods:** 36 meat samples were collected from local markets in Nineveh Governorate. The contamination of various meat samples with enteric bacteria was assessed. The bacteria were subsequently isolated and identified using culture, microscopy, and biochemical techniques. Vitek-2 device was used to verify the diagnosis. **Results:** All of the meats tested were contaminated with enteric bacteria to varied degrees. Fresh chicken meat had the highest rate of enteric bacterial infection, with a logarithm of 1.12×10^8 CFU/g. The frozen beef samples had the least contamination, with a logarithm of 7.4×10^4 CFU/g. The results revealed that 57 isolates from the intestine family bacteria included 13 species: *C. freundii*, *C. koseri*, *E. coli*, *Enterobacter* spp., *K. oxytoca*, *K. pneumoniae*, *P. mirabilis*, *P. vulgaris*, *P. stuartii*, *S. paratyphi A*, *S. typhi*, *Shigella* spp., and *Y. enterocolitica*. *E. coli* was found in the most meat varieties analyzed, accounting for 19% of the total. **Conclusions:** Because it is evident that meat can be contaminated with a wide variety of hazardous bacteria, basic hygiene procedures help to decrease the amount of contaminated microbes.

Keywords: Enteric bacteria, Meat contamination, *E. coli*, Culture media

Introduction

Food-borne illnesses are the most serious international health issue, resulting in economic losses and lives. According to the World Health Organization (WHO), 600 million individuals worldwide are affected by foodborne infections each year. Every year, around 420,000 people die as a result of antibiotic-resistant microorganisms [1]. Meat is one of the most perishable foods and a high-protein source. Meat, on the other hand, poses a high risk of food poisoning since it contains all of the ingredients that promote bacterial growth. As a result, meat, like other perishable goods, is preserved through canning and freezing [2, 3].

The category of enteric bacteria is the most common contamination of meat and its numerous derivatives globally. Not only that, but this bacteria is linked to all occurrences of food poisoning, including *E. coli*, *Proteus* spp, *Salmonella* spp, and *Shigella* spp [4]. Its pathogenicity is primarily determined by its ability to release and secrete numerous toxins, as well as its ability

Comment [FG1]: forms of disease of varying severity up to the death of the consumer

Comment [FG2]: are instead of is

Comment [FG3]: antibiotico resistenza e contaminazione microbica sono 2 cose differenti

Comment [FG4]: sostituire con: enteric bacteria are the main source of contamination

Comment [FG5]: are instead of is

38 to adhere to surfaces, form biofilms, and produce various virulence enzymes, particularly
39 hemolytic and proteolytic enzymes [5]. The manner of preparing meat, the tools used in dealing
40 with it, the personal cleanliness of the employees, and the method of storing and exhibiting it
41 make it susceptible to contamination [6]. Bacterial food poisoning instances are classified into
42 two kinds. The first is a case of food poisoning induced by the presence of bacteria. The other is
43 poisoning from bacteria toxins generated or released in food. Poisoning-related disease
44 symptoms include abdominal discomfort, diarrhea, vomiting, nausea, fever, breathing
45 difficulties, and death in severe situations [7]. The severity and impact of this form of disease are
46 determined by the interplay of the pathogen transmitted by food, the host, the food, and the
47 environment [8].

48 Given the health and economic importance of bacterial contamination of meat sources, as well as
49 the pathogenicity importance of members of the enterobacteriaceae, the current study was
50 designed to assess the prevalence and distribution of different types of contaminated enteric
51 bacteria in various meat and its products.

52 **Materials and methods**

53 36 meat samples were acquired from various sources (fresh beef, fresh lamb, fresh chicken, fresh
54 fish, frozen red meat, frozen pastrami, frozen chicken liver, frozen chicken breast, frozen
55 burger). Meat samples were collected in sterile boxes from local markets in Nineveh
56 Governorate and sent immediately to the laboratory in less than two hours to undertake the initial
57 isolation stages for enteric bacteria.

58 **Isolation and estimation of contamination of meat with enteric bacteria:**

59 10g of meat samples are placed in 90ml of peptone water media (LAB/England). A mixer
60 (Stainless Steel/Chania) crushed it. Using normal saline, 1ml of solution was diluted into
61 multiple decimal dilutions. 1ml of the final dilutions was inoculated on MacConkey agar
62 (LAB/England). The number of colonies was determined by CFU/g. The isolated bacteria were
63 classified based on their capacity to ferment lactose. The microorganisms were cleansed and
64 prepared for further testing [9].

65 **Phenotypic diagnosis:**

66 The diagnosis was made based on the phenotypic characteristics of bacterial colonies in terms of
67 form, color, texture, and fermentation type on MacConkey media. Gram stain was used for
68 microscopic inspection [10].

69 **Biochemical tests:**

70 All isolates were subjected to the following biochemical [11] tests: Oxidase, indol, methyl red
71 (MR)/Voges-Proskauer (VP), citrate utilization, triglyceride iron test (TSI), and Urease test. The
72 Vitek-2 system was used for confirmatory diagnostic testing.

73 **Results and discussion**

Comment [FG6]: contaminating instead of contaminated

Comment [FG7]: types instead of sources

Comment [FG8]: In my opinion it is advisable to know, to understand the significance of the sampling and the observed results, the subdivision of the 36 samples analyzed into the different types of meat: how many samples were fresh beef? How many were fresh fish? and so on. This information could be added in table 1

74 The results revealed a clear variance in the extent of contamination with different meat sources,
 75 as indicated in Table (1) and Figure (1, 2). The manner of preparing meats, the tools used in
 76 dealing with them, the personal cleanliness of the employees, and the method of keeping and
 77 presenting them may be the source of contamination of different meats with different enteric
 78 bacteria. As a result, it becomes an ideal environment for bacterial reproduction [12].

Comment [FG9]: in my opinion, the hygiene of the slaughterhouse should also be considered as a possible initial source of contamination

79 According to the study, fresh chicken meat had the highest percentage of enteric bacterial
 80 infection, with a logarithm of 1.12×10^8 CFU/g. The high pollution rate might be attributed to the
 81 frequent use of the chicken feather removal device and the failure to wash it after extended
 82 periods of time, as well as the failure to change the water for long durations of time. Fresh fish
 83 had the second highest contamination rate of 1.16×10^7 CFU/g, which could be attributed to the
 84 placing of fish in dirty water ponds, the frequent use of these ponds, and the lack of water
 85 exchange for extended periods of time. These ponds are thought to be an ideal environment for
 86 the growth of microorganisms.

Comment [FG10]: this instead of the

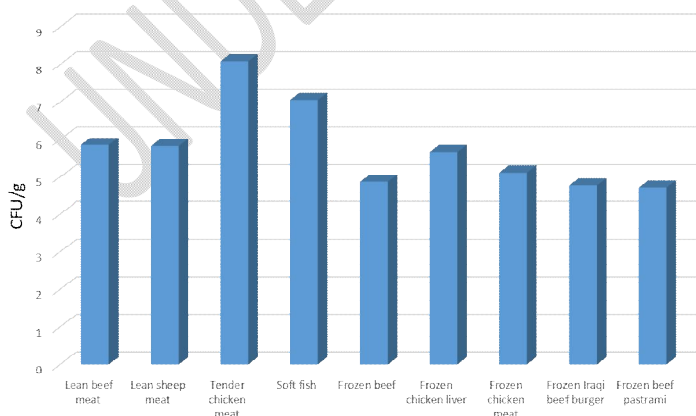
Comment [FG11]: this does not seem to me a great consideration, in any study the contamination of chicken meat is higher than that of bovine, sheep and goat or other species. look for some supporting bibliographic sources

87 **Table 1:** Average number of bacterial colonies (CFU/g) in different meat samples

Sample source	Average no. of colonies
Fresh beef meat	7.2×10^5
Fresh sheep meat	6.8×10^5
Fresh chicken meat	1.12×10^8
Fresh fish	1.16×10^7
Frozen beef	7.4×10^4
Frozen chicken liver	4.7×10^5
Frozen chicken meat	1.31×10^5
Frozen Iraqi beef burger	5.8×10^4
Frozen beef pastrami	5.1×10^4

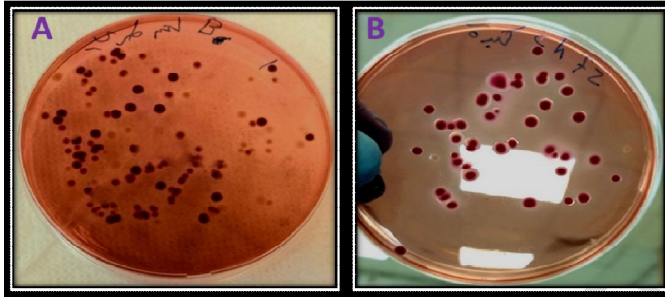
Comment [FG12]:

88



89

90 **Fig 1:** Contamination percentages in different meat sources log₁₀(CFU)/g of enterobacteria.



91
92 **Fig 2:** Models representing the extent of contamination with enteric bacteria on McConkey agar media in different
93 meat samples. A: Showing a sample of fresh beef from (the third dilution). B: Result of culturing a frozen chicken
94 liver sample from (the third dilution).

95 **Prevalence and distribution of enteric bacteria:**

96 **Enterobacteriaceae** were isolated by using MacConkey agar media divided based on their ability
97 to ferment lactose (pink colonies) and non-lactose-fermenting bacteria (transparent colonies)
98 [10]. **Enterobacteriaceae** isolates of meat sources identified based on the agronomic,
99 microscopic, and biochemical features mentioned in Table (2), as well as confirmation of the
100 isolates' diagnosis by using Vitek-2. Isolated bacteria were dispersed among 13 distinct species,
101 which included: *C. freundii*, *C. koseri*, *E.coli*, *Enterobacter* spp., *K. oxytoca*, *K. pneumoniae*, *P.*
102 *mirabilis*, *P. vulgaris*, *P. stuartii*, *S.paratyphi A*, *S. typhi*, spp. *Shigella* and *Y. enterocolitica*. All
103 bacterial types were isolated from fresh chicken, fresh beef, fresh lamb, fresh fish, frozen red
104 meat, frozen pastrami meat, frozen chicken breast, frozen chicken liver, and frozen burger.

105 **Table 2:** Biochemical tests of bacteria isolated from different meat sources.

Bacteria Sp	Oxidase	Urease	TSI Test			IMViC Test			
			H ₂ S	Gas	S/B	CIT	VP	MR	IND
<i>C. koseri</i>	-	-	-	+	A/A	+	-	+	+
<i>E.coli</i>	-	-	-	+	A/A	-	-	+	+
<i>P. vulgaris</i>	-	+	+	-	K/A	-	-	+	+
<i>P. mirabilis</i>	-	+	+	+	K/A	+	-	+	-
<i>Enterobacter</i>	-	+	-	+	A/A	+	+	-	-
<i>P. stuartii</i>	-	-	-	+	K/A	+	-	+	+
<i>Shigella</i>	-	-	-	-	K/A	-	-	+	-
<i>C. freundii</i>	-	-	+	+	A/A	+	-	+	-
<i>S.paratyphi A</i>	-	-	-	+	K/A	-	-	+	-
<i>S. typhi</i>	-	-	+	-	K/A	-	-	+	-
<i>Y. enterocolitica</i>	-	+	-	-	K/A	-	-	+	-
<i>K. pneumoniae</i>	-	+	-	+	A/A	+	+	-	-
<i>K. oxytoca</i>	-	+	-	+	A/A	+	+	-	+

Comment [FG13]: in my opinion, inserting 2 figures of culture plates is useless, one of the 2 is enough: choose the best one, nor is it useful to specify at which dilution the bacterial growth photographed was obtained

Comment [FG14]: change with are reported

Comment [FG15]: this sentence is confusing, it does not give any information about the source of isolation of the different microorganisms

106 TSI: Triple Sugar Iron, S/B: butt/slant, Gas: gas production, A: acid, K: alkaline, IND: Indole, MR: Methyl red, VP:
 107 Voges–Proskauer, CIT: Citrate.

108 ~~Because most~~ isolated bacteria are harmful, they cause diseases such as intestinal infections,
 109 urinary tract infections, pneumonia, meningitis, and sepsis. The majority of them also produce
 110 intestinal toxins, which induce food poisoning [13]. Clinically, the majority of gut bacteria
 111 genera are categorized as opportunistic infections. Some of them are classed as basic pathogens,
 112 such as *Salmonella*, *Shigella*, and *Yersinia*, as well as being one of the most common food-borne
 113 pathogens, particularly *E. coli* bacteria [4]. These are the species that naturally inhabit the human
 114 intestine and spread easily by hand contamination, water, and food. Treatment and control are
 115 difficult, especially in low-income nations [14].

Comment [FG16]: some of the bacteria isolated and identified are harmful, and they can

Comment [FG17]: enter "could"

116 The results revealed a difference in the presence of the isolated enteric bacterial species, which
 117 implies variance in the size and kind of contamination. Some meats had only two species (frozen
 118 chicken liver), whereas others contained seven types (fresh beef). The remaining samples
 119 contained 6-3 enteric bacteria, according to the Table (3). *E. coli* was the most common from
 120 different isolate sources (6 sources), reflecting the extensive dissemination. This was followed by
 121 *C. koseri*, *Enterobacter* spp (5 sources), and *Proteus* (4 sources). In terms of the number and
 122 percentage of isolates, it was discovered that *E. coli* had the highest isolates 19%, followed by *C.*
 123 *koseri* 16%, and *Proteus mirabilis* 12.3%. *Enterobacter* spp. and *Shigella* spp. each accounted
 124 for 10.5% of the total, Figure (3).

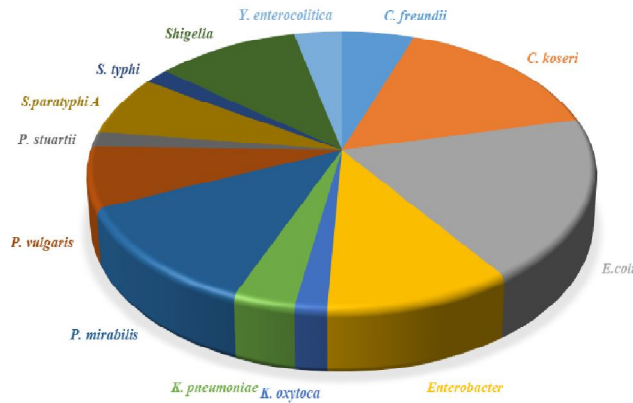
Comment [FG18]: the lack of information on how many samples were made for the different types of meat (36 samples, 9 types means 4 samples per type: very few to make general considerations) makes this discussion useless in my opinion

125 **Table 3:** Types of enteric bacteria isolated from different meat sources.

Source of sample	No. of isolates	Bacteria (no.)
Fresh chicken	6	<i>Citrobacter koseri</i> (2), <i>E. coli</i> (1)
		<i>Enterobacter spp</i> (1), <i>Proteus mirabilis</i> (1)
		<i>Proteus vulgaris</i> (1)
Fresh beef meat	13	<i>Citrobacter koseri</i> (2), <i>E. coli</i> (2)
		<i>Enterobacter spp</i> (1), <i>Proteus mirabilis</i> (4)
		<i>Proteus vulgaris</i> (1), <i>Providencia stuartii</i> (1)
		<i>Shigella spp</i> (2)
Fresh sheep meat	7	<i>E. coli</i> (4), <i>Enterobacter spp</i> (1)
		<i>Proteus mirabilis</i> (2)
Fresh fish	4	<i>Citrobacter freundii</i> (1), <i>E. coli</i> (1)
		<i>S.paratyphi A</i> (1), <i>Shigella spp</i> (1)
Frozen red meat	10	<i>Citrobacter koseri</i> (1), <i>E. coli</i> (2)
		<i>Enterobacter spp</i> (2), <i>Salmonella typhi</i> (1)
		<i>Shigella spp</i> (2), <i>Yersinia enterocolitica</i> (2)
Frozen beef pastrami	3	<i>E.coli</i> (1), <i>Proteus mirabilis</i> (1)
		<i>S.paratyphi A</i> (1)
Frozen chicken liver	3	<i>Citrobacter koseri</i> (2), <i>Klebsiella pneumoniae</i> (1)
Frozen breast chicken	8	<i>Citrobacter freundii</i> (2), <i>Citrobacter koseri</i> (2), <i>Klebsiella oxytoca</i> (1), <i>Proteus vulgaris</i> (1) <i>S.paratyphi A</i> (2)

126

Frozen beef burger	3	<i>Enterobacter spp(1), Shigella spp(1)</i>
		<i>Klebsiella pneumoniae(1)</i>



127

128 **Fig 3:** The proportions of isolating types of enterobacteria from the studied meat sources.

129

130 Members of the enteric family play a significant role in the transmission of foodborne illnesses.
 131 Foodborne infections continue to be a real and major health and economic hazard around the
 132 world [1]. According to the report of WHO in 2021, 600 million people worldwide suffer from
 133 foodborne infections each year, and 420,000 people die as a result of foodborne diseases,
 134 particularly antibiotic-resistant bacteria. [The] spread is frequently caused by fecal contamination
 135 of food sources, which contain several bacterial species [15].

136 **Conclusions**

137 Because it is obvious that meat can be infected with a wide range of dangerous bacteria, good
 138 hygiene procedures help to limit the quantity of contaminated germs. Furthermore, a
 139 commitment to take precautions when cutting and preserving meat, as well as applying sound
 140 manufacturing processes, is required to keep meat contamination to an acceptable level. Future
 141 research should concentrate on the precise characterization of the population structure of
 142 foodborne pathogens in order to better understand epidemiology, pathogenicity, and antibiotic
 143 resistance profiles.

144 **References**

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Comment [FG19]: the problem of antibiotic resistant bacterial strains is mentioned but this evaluation was not made on the isolates of this study and therefore the reference appears meaningless

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