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

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Original Research Article

ABSTRACT

Food-borne diseases are the most serious international health issue, causing economic losses and health. 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.

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1. INTRODUCTION

Food-borne illnesses are the most serious international health issue, resulting in economic losses and health. 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 bacteria [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 frequent meat contaminant, and its numbers are increasing internationally. Not only that, but these bacteria are 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 to adhere to surfaces, form biofilms, and produce various virulence enzymes, particularly hemolytic and proteolytic enzymes [5]. The manner of preparing meat, the tools used in dealing with it, the personal cleanliness of the employees, and the method of storing and exhibiting it make it susceptible to contamination [6]. Bacterial food poisoning instances are classified into two kinds. The first is a case of food poisoning induced by the presence of bacteria. The other is poisoning from bacteria toxins generated or released in food. Poisoning-related disease symptoms include abdominal discomfort, diarrhea, vomiting, nausea, fever, breathing difficulties, and death in severe situations [7]. The severity and impact of this form of disease are determined by the interplay of the pathogen transmitted by food, the host, the food, and the environment [8].

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

2. MATERIALS AND METHODS

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

Isolation and estimation of contamination of meat with enteric bacteria:

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

Phenotypic diagnosis:

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

Biochemical tests:

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

3. RESULTS AND DISCUSSION

The results revealed a clear variance in the extent of contamination with different meat sources, as indicated in Table 1 and Figs. 1, 2. The manner of preparing meats, the tools used in dealing with them, the personal cleanliness of the employees, and the method of keeping and presenting them may be the source of contamination of different meats with different

enteric bacteria. As a result, it becomes an ideal environment for bacterial reproduction [12].

According to this study, fresh chicken meat had the highest percentage of enteric bacterial infection, with a logarithm of $1.12 * 10^8$ CFU/g. The high pollution rate might be attributed to the frequent use of the chicken feather removal device and the failure to wash it after extended periods of time, as well as the failure to change the water for long durations of time. Fresh fish had the second highest contamination rate of $1.16 * 10^7$ CFU/g, which could be attributed to the placing of fish in dirty water ponds, the frequent use of these ponds, and the lack of water exchange for extended periods of time. These ponds are thought to be an ideal environment for the growth of microorganisms [11].

Prevalence and distribution of enteric bacteria:

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

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

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

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

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

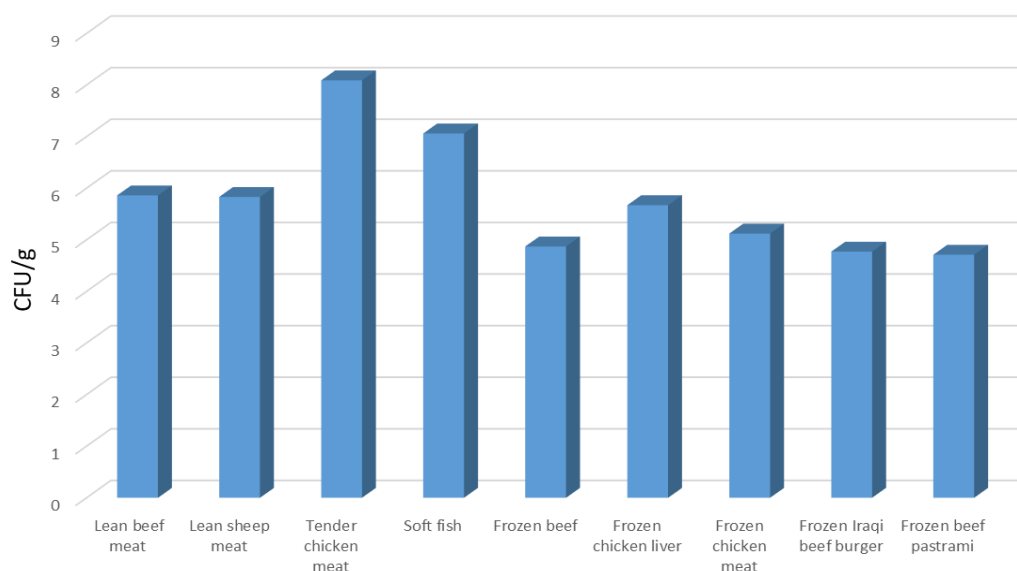| Sample source	Average no. of colonies (CFU/g)
Fresh beef meat	$7.2 * 10^5$
Fresh sheep meat	$6.8 * 10^5$
Fresh chicken meat	$1.12 * 10^8$
Fresh fish	$1.16 * 10^7$
Frozen beef	$7.4 * 10^4$
Frozen chicken liver	$4.7 * 10^5$
Frozen chicken meat	$1.31 * 10^5$
Frozen Iraqi beef burger	$5.8 * 10^4$
Frozen beef pastrami	$5.1 * 10^4$

Fig. 1. Contamination percentages in different meat sources log 10(CFU)/g of enterobacteria
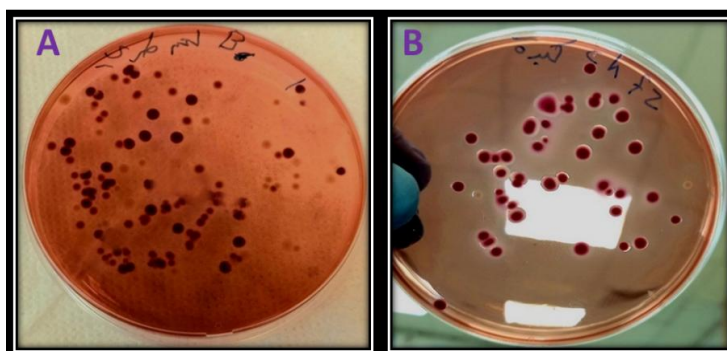

Fig. 2. Models representing the extent of contamination with enteric bacteria on McConkey agar media in different meat samples. A: Showing a sample of fresh beef from (the third dilution). B: Result of culturing a frozen chicken liver sample from (the third dilution)

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

Bacteria Sp	Oxidase	Urease	TSI test			IMViC test			
			H₂S	Gas	S/B	CIT	VP	MR	IND
C. koseri	-	-	-	+	A/A	+	-	+	+
E. coli	-	-	-	+	A/A	-	-	+	+
P. vulgaris	-	+	+	-	K/A	-	-	+	+
P. mirabilis	-	+	+	+	K/A	+	-	+	-
Enterobacter	-	+	-	+	A/A	+	+	-	-
P. stuartii	-	-	-	+	K/A	+	-	+	+
Shigella	-	-	-	-	K/A	-	-	+	-
C. freundii	-	-	+	+	A/A	+	-	+	-
S. paratyphi A	-	-	-	+	K/A	-	-	+	-
S. typhi	-	-	+	-	K/A	-	-	+	-
Y. enterocolitica	-	+	-	-	K/A	-	-	+	-
K. pneumoniae	-	+	-	+	A/A	+	+	-	-
K. oxytoca	-	+	-	+	A/A	+	+	-	+

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

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

| Source of sample	No. of isolates	Bacteria (no.)
Fresh chicken	6	*Citrobacter koseri (2), E. coli (1) Enterobacter spp (1), Proteus mirabilis (1) Proteus vulgaris (1)*
Fresh beef meat	13	*Citrobacter koseri (2), E. coli (2) Enterobacter spp (1), Proteus mirabilis (4) Proteus vulgaris (1), Providencia stuartii (1) Shigella spp (2)*
Fresh sheep meat	7	*E. coli (4), Enterobacter spp (1) Proteus mirabilis (2)*
Fresh fish	4	*Citrobacter freundii (1), E. coli (1) S. paratyphi A (1), Shigella spp (1)*
Frozen red meat	10	*Citrobacter koseri (1), E. coli (2) Enterobacter spp (2), Salmonella typhi (1) Shigella spp (2), Yersinia enterocolitica (2)*
Frozen beef pastrami	3	*E. coli (1), Proteus mirabilis(1) S. paratyphi A (1)*
Frozen chicken liver	3	*Citrobacter koseri (2), Klebsiella pneumoniae (1)*
Frozen breast chicken	8	*Citrobacter freundii (2), Citrobacter koseri (2), Klebsiella oxytoca (1), Proteus vulgaris (1) S. paratyphi A (2)*
Frozen beef burger	3	*Enterobacter spp (1), Shigella spp (1) Klebsiella pneumoniae (1)*
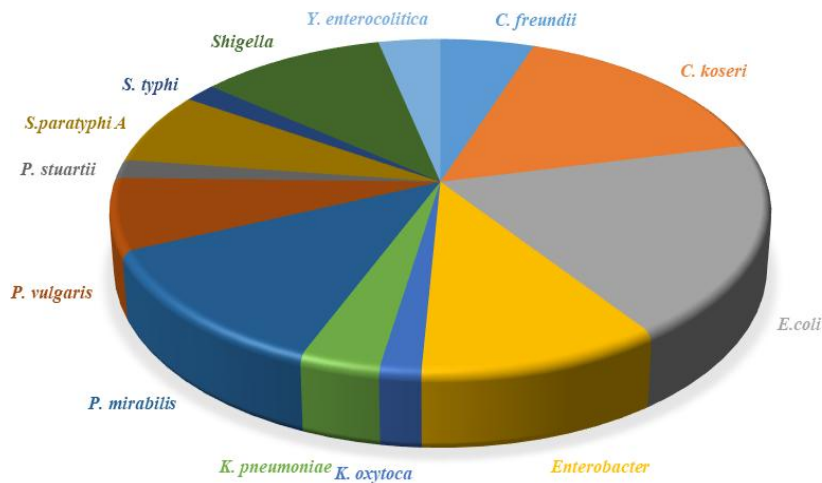
Fig. 3. Proportions of isolating types of enterobacteria from the studied meat sources

4. CONCLUSIONS

Because it is evident that meat can be contaminated with a variety of hazardous bacteria, basic hygiene techniques serve to decrease the number of contaminated germs. Furthermore, to prevent meat contamination to an acceptable level, a dedication to taking precautions when cutting and storing meat, as well as using good production procedures, is necessary. Future research should focus on the precise characterization of foodborne pathogen population structure in order to better understand epidemiology, pathogenicity, and antibiotic resistance profiles.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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