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Occurrence of Enterobacteriaceae in Raw Chicken Meat Samples with Identification of *Salmonella enterica* subsp. *Diarizonae* as the First Report in Iraq

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Abstract

The present study was performed to assess the presence of Enterobacteriaceae in raw meat in Iraq using cultivation and the VITEK®2 compact system. A total of 20 chicken raw meat samples were randomly purchased from butchers and local meat retailers located in Najaf, Iraq. Five bacterial isolates were recovered from these samples. *Proteus mirabilis* (70%) was found to be the most abundant, followed by *Enterobacter cloacae complex* (15%), *Pseudomonas aeruginosa* and *Salmonella enterica* subsp. *diarizonae* (10%), and *Enterobacter aerogenes* (5%). The discovery of *Salmonella enterica* ssp. *diarizonae* in local chicken meat is the first of its kind in Iraq. Moreover, the presence of several Enterobacteriaceae in locally produced retail raw chicken meat raises concerns about the possibility of cross-contamination with other food items. Also enhances the danger of human infection from eating raw or undercooked meat. To lessen the danger of infection, veterinarians and public health authorities must coordinate and take synchronized action.

Keywords

Chicken meat

Enterobacteriaceae

Salmonella enterica subsp. *Diarizonae*

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Introduction

Chicken meat is considered to be of high nutritional value, as it contains proteins, amino acids, and vitamins necessary for humans. In addition, it is easy to prepare and digest and is suitable for all age groups (Gwida *et al.*, 2014). Many consumers prefer poultry meat over red meat from a medical point of view, as its calorie content is less than that of red meat, which ranges between 117 and 130 calories in comparison with beef, which ranges from 180 to 320 calories. Besides that, due to its low fat content, poultry meat is suitable for feeding disease-recovery patients. Poultry meat also contains 23.4% protein, 73.8% water, and a pH of 5.7 to 6.2 (Anning *et al.*, 2019). It is noted that the percentage of unsaturated fatty acids in this type of meat is high, as fats that contain a high percentage of saturated fatty acids help to deposit cholesterol (Areekit *et al.*, 2019). Uninfected chicken meat is free from microbial contamination, but the contamination occurs once the first step of the fresh meat production process is started. Usually, the animal is the source of pollution or its external surroundings, as contamination occurs first on the external surface of the meat, and then contamination increases with processing and marketing operations. Consequently, low-quality chicken is a poultry source responsible for human infection with various diseases (Benameur *et al.*, 2018). Several studies have found that the most important bacterial species isolated from chicken meat are *Pseudomonas, Salmonella, E. coli, S. aureus* and *Listeria* (Anning *et al.*, 2019).

Moreover, *salmonella* has been detected in carcasses of chickens due to the high rate of contamination with the feces of infected birds during processing in slaughterhouses (Siriken *et al.*, 2015). The number of bacteria found mainly in chicken meat is a result of inadequate preparations and handling in slaughterhouses, and the more this number increases, the less time the carcasses remain fit for consumption (Ji *et al.*, 2021). Most of these bacteria reach the food of infected people and contaminated hands of workers, from wounds, respiratory tracts, and surrounding work (Anning *et al.*, 2019). Owners of chicken processing

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houses have the main responsibility of completing several steps related to hygiene, and not only adding preservatives to assure that the manufactured food will not cause harm to the consumer's health (Gwida *et al.*, 2014). Otherwise, the withdrawal of contaminated food products from the market may lead to a reduction in the companies' productivity and deteriorating public health (Al-Subeihi, 2022).

Lacking of monitoring policies of chicken meat handling due to the weakness of the control, standardization, and quality control organizations in developing countries such as Iraq, where political and economic issues have been established. Therefore, this study was directed to assessing the quality and microbial quantity of local chicken meat found in the local markets to determine its suitability for human consumption.

Materials and methods

20 samples of chicken meat were collected from the different areas of several small and large shops in the city of Najaf. The samples were transferred under cooled and sterilized conditions to the Microbiology Laboratory of the College of Agriculture / University of Kufa. 50 g of each sample were taken from different areas and divided into two parts. Every 25 g was placed in a blender previously sterilized with hot water and ethanol alcohol 95%. The mixture was blended with 225 mL buffer peptone water for 2 min, then left for 20 min to activate the bacteria to obtain a concentration of 0.1 mL of a homogeneous solution of the sample.

Total bacterial count

Transfer 1 mL of the homogenized solution (containing a 0.1 mL concentration of the sample) to a tube containing 9 mL of normal saline. To obtain a concentration of 0.01, transfer 1 mL of the last dilution to a petri dish and pour 15-20 mL of nutrient agar. Three replicates were made for each sample. The plates were incubated with the control without any samples at 37 °C for 24 h (Mariya *et al.*, 2020).

Isolation of Enterobacteriaceae

Then 1 mL of the last dilution was transferred to a Petri dish (three replicates of each sample) and MacConkey agar was poured over. The plates were incubated with the control plate at 37 °C for 24 h and the growth of bacterial colonies was monitored (Mariya *et al.*, 2020).

Salmonella isolation

The samples were cultured on a Bismuth sulfate medium for the determination of *Salmonella*'s distinctive black colonies.

Diagnosis of samples using the VITEK®2 compact system

The bacteria suspension was prepared according to the manufacturer's instructions to obtain a sufficient number of cells using the VITEK®2 compact system (bioMérieux, Inc/US). In a sterile saline solution, a single colony of oxidase-positive, gram-negative rods (20 samples) was suspended. The turbidity was adjusted to 0.5 using a McFarland tube using a turbidity meter, and then the suspension was placed into a TDR-300B NF-64 card and VITEK®2 GN cassette for each sample. Finally, bacterial identification was performed using VITEK®-MS for comparative analysis (Sobhy & Shaltout, 2020).

Results and discussion

In this study, 20 samples of chicken meat were collected from various local stores in Najaf city. The samples were cultivated and the phenotypic characteristics were primarily and presumptively assessed. The black colonies of *Salmonella enterica* subsp *diarizonae* are shown in Fig. (1). The isolates were further processed for confirmation of the identification using the VITEK® 2 compact system VITEK® 2 compact system. The results revealed the appearance of 4 bacterial genera represented by *Proteus mirabilis* (99% probability), *Pseudomonas aeruginosa* (97% probability), *Enterobacter cloacae* complex (99% probability), *Salmonella enterica* subsp. *diarizonae* (95% probability) and *Enterobacter aerogenes* (87% probability), Fig. (2). Twelve bacterial isolates out of 20 (70%) samples were identified as *P. mirabilis* from chicken meat, and this result was noteworthy. *E. cloacae* were isolated inappropriate and absence of monitoring of from 3 samples (15%), followed by 2 samples (10%) of *P. aeruginosa*.

Salmonella isolation

Based on the existing research, this is the first report to identify *Salmonella enterica* subsp. *diarizonae* in Iraq (Fig. 2). This isolate was found in 2 samples out of 20 samples, while *E. aerogenes* was detected in only one sample (5%), (Table 1).

Table 1: Identification of bacterial isolates in chicken meat by VITEK® 2 compact

Identified microorganism	Raw chicken meat (n = 20)	Occurrence rate %
Proteus mirabilis	12	70
Enterobacter cloacae complex	3	15
Pseudomonas aeruginosa	2	10
Salmonella enterica ssp. diarizonae	2	10
Enterobacter aerogenes	1	5
Total number of isolates	20	

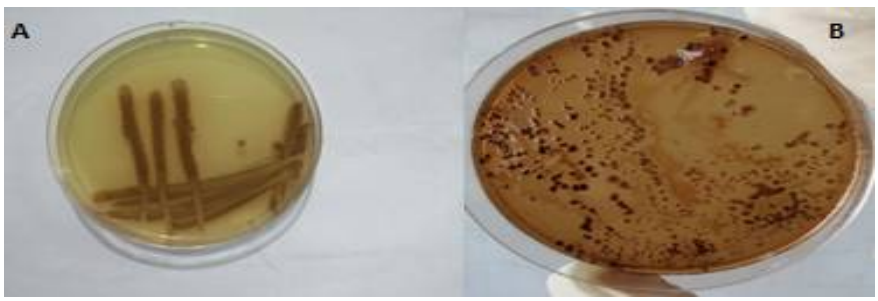

Fig. 1. Primarily diagnosis of the *Salmonella enterica* subsp *diarizonae* isolate is based on the phenotypic characteristics on different media; (A) *Salmonella enterica* subsp. *diarizonae* was found growing on Bismuth sulfate agar, (B) *Salmonella enterica* subsp. *diarizonae* on MacConcky agar.

Selected Organism	99% Probability Bionumber:	Proteus mirabilis 0013000301442210
ID Analysis Messages		

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	-
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	-	21	BXYL	-	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	+	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	-	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	+
46	GlyA	-	47	ODC	+	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Selected Organism	99% Probability Bionumber:	Enterobacter cloacae complex 0627634553533010
ID Analysis Messages		

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	+	44	AGAL	+	45	PHOS	-
46	GlyA	+	47	ODC	+	48	LDC	-	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Selected Organism	97% Probability Bionumber:	Pseudomonas aeruginosa 0043453143500250
ID Analysis Messages		

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	+
23	ProA	+	26	LIP	+	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	+	62	ELLM	-	64	lLATa	-			

Selected Organism	95% Probability Bionumber:	Salmonella enterica ssp diarizonae 0417651543526610
ID Analysis Messages		

Biochemical Details																
2	APPA	-	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	-	9	BGAL	+
10	H2S	+	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	-	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	-	22	BAlap	+
23	ProA	+	26	LIP	-	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	-	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	-
46	GlyA	-	47	ODC	+	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Selected Organism	87% Probability Bionumber:	Enterobacter aerogenes 1627737453466610
ID Analysis Messages		

Biochemical Details																
2	APPA	+	3	ADO	-	4	PyrA	-	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	+	26	LIP	+	27	PLE	+	29	TyrA	-	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	-	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	+	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	+
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	lLATa	-			

Fig. 2. The confirmation results for bacterial isolates identity using VITEK® 2 compact.

In this study, the high percentage occurrence of *P. mirables* was 70%, and it is a high number of 12 samples out of 20 samples Table (1). *P. mirablis* strains were isolated from raw chicken meat samples from open markets in Bangkok, Thailand, and the genome sequence of *P. mirabilis* virulence factors was determined. The genes of multidrug efflux pumps were recognized as virulence factors and enhanced the antibiotic resistance of bacteria (Areekit *et al.*, 2019). The incidence of *P. mirabils* in the chicken meat samples can be ascribed to unhygienic behaviors during the chicken meat's raw handling. This isolate was detected by researchers from contaminated chicken meat and its products (Gwida *et al.*, 2014; Schaffer & Pearson, 2015). Furthermore, it has been established that *Proteus* spp. is a concern and threat to the public's health and can cause diarrhea and urinary tract infections *(*Anning *et al.*, 2019*;* Schaffer & Pearson, 2015*)*. Three samples out of 20 (15%) were identified as *E. cloacae* and two samples (10%) yielded *P. aeruginosa* (Table 1, Fig. 2). The incidence of Enterobacteriaceae may be due to the contact of the surface of the carcass with blood during the

slaughter process, especially with the clear reservation in the use of water in washing. Moreover, the occurrence of cross-contamination resulting from contact with people and the use of the same equipment, such as knives, in all stages of the processing operations without washing them continuously, or washing them in basins with cold water does not contain any disinfectants, and contaminated water is not changed until after a long period (Chen *et al.*, 2020; Gwida *et al.*, 2014). Benameur *et al.* (2018) isolated *E. cloaca* 52 (94.54%) from poultry in Algeria. These outcomes are highly different from the current results. Moreover, *P. aeruginosa* infections in humans have been linked to occupational exposure to poultry carcasses or related products in numerous investigations. *P. aeruginosa* is regarded as a significant spoiling agent found in ruined poultry meat offered for sale in retail establishments (Chen *et al.*, 2020; Gong *et al.*, 2018; Morales *et al.*, 2016). Chicken meat contamination may happen during the evisceration or slaughter of an animal due to spillage of fecal material from rupture of the gut. Food handlers may contaminate their hands with bacteria from either their stool or during carcass handling (Ji *et al.*, 2021). This is clear from the above results that chicken meat contained *Salmonella enterica* spp. *dirazonae* is the first report on the identification of this isolate in Iraq. Typically, *Salmonella enterica* subsp. *dirazonae* is isolated from the environment, cold-blooded reptiles, sheep, and humans. However, (Pławińska-Czarnak *et al.*, 2022) isolated this subspecies from poultry meat, which is comparable to current results. The researchers discovered the first detection of *Salmonella enterica* subsp. *diarizonae* in Poland in a wild duck (*Anas platyrhynchos*). During the winter, this species of bird migrates from Europe to Iraq. Thus, *Salmonella enterica* subsp. *dirazonae* transmission to Iraq might be occurred through birds migration season.

Conclusion

To conclude with the detection of the presence of Enterobacteriaceae in raw meat in Iraq using cultivation and the VITEK®2 compact system. The results revealed Enterobacteriaceae isolates in chicken meat and the first detection of *Salmonella enterica* subsp. *diarizonae* isolates in Iraq. Detection of this isolate suggests the cross-border environment and increases the risk of human infection by consuming the chicken without proper handling and cooking. the application of well-organized precautionary measures at all levels of preparation of chicken meat from farm to consumer table is becoming mandatory. Moreover, additional investigations such as the antibiotic resistance of this new strain should be accomplished. Therefore, identification of precautions procedures need to be undertaken so that minimizing the consequences of this bacteria and its virulence ability.

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Author contributions

Ameer Salem El-Esawi: Data analysis, Writing the draft of the manuscript, Supervising the study, Approval of the final version; **Zeina Taleb Al-Salami**: Presenting the research idea and study design, Approval of the final version; **Salah Mahdi Al-Jannah**: Data analysis and interpretation, Presenting the research idea and study design, Approval of the final version; **Khawlah Abdallah Salman**: Data collection, Writing the draft of the manuscript, Revising and editing the manuscript, Approval of the final version.

Conflict of interest

The authors declare that there is no conflict of interest.

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