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ISOLATION AND IDENTIFICATION OF ZOONOTIC IMPORTANCE BACTERIA FROM MEAT, MEAT PRODUCTS AND HUMAN IN DIYALA, IRAQ

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Abstract

To study the common bacteria of zoonotic importance, contaminated meat and meat products, a total of 251 samples represent (35) raw meat and meat products from cow and sheep, (41) from poultry, in addition to (175) swabs from workers and their equipment used in shops, restaurants, were collected from August, 2019 to April, 2020. The samples were submitted to laboratory examination, included isolations and identifications of isolates. The results revealed from 251 samples, (328) isolates were isolated. The common isolates were *Staphylococcus* sp. 77/328 (23.5%); followed by *Klebsiela* sp. 66/328 (20.1%); *E. coli* 50/328 (15.2%), *Pseudomonas* sp. 44/328 (13.4%); *Salmonella* sp. 19/328 (5.8%); *Listeria* sp.16/328 (4.9%); *Proteus* and *Citrobacter* each 14/328 (4.3%), *Enterobacter* 13/328(4.0%), *Yersinia* sp., 8/328 (2.4%); *Streptococcus* 6/328 (1.8%) and *Shigella* sp. 1/328 (0.3%). From 175 samples collected from workers and their equipment. 197 isolates were isolated. The highest isolate was Kleb.44/197 (22.3%); followed by Staph. 42/197 (21.3%); *E. coli* 34/197 (17.3%); Pseud. 26/197 (13.2%); Prot. 14/197(7.1%); Yersinia and Cit. each 8/197(4.1 %); Ent. 7/197 (3.6%); Strept. 6/197(3.0%); Sal. 4/197 (2.0%); list. 3/197 (1.5%); Shigella, 1/197(0.5%). From 15 sheep's meat and meat products; 25 isolates were isolated The Staph. was isolated in highest numbers 8/25 (32.0%); pseud 5/25 (20.0%); Kleb. 4/25 (16.0%); List. and *E. coli* 3/25 (12.0%); Sal. 2/25 (8.0%). From 20 beef and beef products, 31 isolates were isolated. The highest number of isolates was Staph. 11/31 (35.5%); Kleb. 9/31 (29.0%); Pseud. 4/31 (12.9%); List 3/31 (9.8%); *E. coli*; Sal.; Cit. and Ent. 1/31 (3.2%). From 41 poultry's meat and meaty products, 75 isolates were isolated. Staph 16/75 (21.3%); Sal. and *E. coli* 12/75 (16.0%); Kleb. and Pseud. 9/75 (12.0%); List. 7/75 (9.3%); Cit. and Ent. 5/75 (6.7%). The highest number of isolates were from workers (78.5%), then from poultry meat and meat products (29.9%) and from sheep and calf meat and meaty products (22.3%). Total isolates were 328, from which 141 (43.0%) isolates were in single form and other 187 (57%) in more than one isolates.

Key word: zoonotic importance bacteria, meat products, Human

Introduction

Meat from healthy animals is free of microorganisms. Cross contamination occurs during the handling of raw meat, especially poultry meat, because of the presence of more liquid in raw meat than cooked meat, (Javadi and Saeid, 2011; Koffi-Nevry *et al.,* 2011; Darshana, *et al.,* 2014). The slaughter house usually has a variety of sources of meat contamination with bacteria, as skin, hair, hooves and attached soil, in addition to contents of the digestive tract. fecal materials are a major source of contamination via direct deposition and indirect contact through contaminated tools used in slaughtering, cutting and transport, besides people working in the slaughterhouses (Borch and Arnder, 2002; Mead, 2004; Salihu *et al.,* 2010; Kumar *et al.,* 2012; Kumar, *et al.,* 2014; Keshab, 2015). Contaminated raw meat, causes 90% of food- borne illness (Barbudhe, *et al.,* 2003; Bhandare, *et al.,* 2007; Podpeèan, *et al.,* 2007; Arul and Saravanan, 2014).

The refrigerated poultry meat would be spoilage when stored for a long period due to the microorganism actions in addition to the biochemical transformations inside the product (Octavian, *et al.,* 2010; Al-jasser, M.S., 2012). In Iraq, poultry slaughtered manually, therefor, contaminated by different types of microorganisms bacteria, fungus even parasites from soil or from contaminated earth with other poultry wastes (Siddiqui *et al.,* 2008; Anjum *et al.,* 2004). Sometimes the washing is unbeneficial to be pure from microorganisms (Siddiqui, *et al.,* 2006). While the frozen poultry meat which comes

to markets from unknown origins may be contaminated by certain microbial pollutants, because unknown sources, processing and transporting in addition to instability of electrical power. The aims of study are 1. Isolate the most common bacterial, from meat and meat products from sheep, beef and poultry, workers and their equipment and 2. Identify these isolates.

Materials and Methods

Preparation of Culture Media

All culture media were prepared according to instruction of manufactures corporation and sterilization were completed by autoclaving at (121°C, 15 pound/inch^2 for 15 min). Then poured into a sterilized Petri dishes and/or tubes according to requisite and incubated for 24 hr. at 37°C to ensure sterilization and kept at 4°C until use (Quinn *et al.,* 2004).

Samples

The study was conducted in Department of Medicine, College of Veterinary Medicine, University of Diyala, Iraq, on (251) samples; represent (35) samples raw meat and meat products, obtained from sheep and cattle, (41) samples meat and meat products obtained from poultry and (175) samples obtained from workers in shops and restaurant, with their equipment's and machines, from different places in Diyala province, during the period from Auygust, 2019 to April 2020. Samples were submitted to routine procedures of isolation, identification.

Fresh minced meat samples were purchased from butchers shop, from backyard slaughter, street meat sales shops. The samples were collected aseptically in clean polyethylene bag and transported to the laboratory in icebox. (Cheesbrough,1984). for further bacteriological analysis as described by (Fawole, M.O. & Oso, B.A. 2001).

25g of each sample was treated with 225ml of peptone water and homogenized using a blender for 60s at room temperature.

Bacterial isolation and Identification

The isolates were carefully examined macroscopically by comparing their morphological characteristics such as shape, size, elevation, form, edge, consistency, color, odor, opacity, hemolysis and pigmentation hence result was recorded. Gram staining as well as appropriate biochemical characteristics were carried out according to standard procedures, (Oyeleke, S.B., and Manga, S.B., 2008). With standard reference organisms with those of known taxa, as described by (Buchanan, R.E. and Gibbons, N.E.B. 1974).

Several dilutions were achieved up to fold (10^{-5}) for each prepared samples using 1 ml from stock homogenate and 9 ml of sterile distilled water for the serial dilution experiment.

Plating and culture on media:

0.1 ml was taken from 1-5 folds dilution and dispensed in sterile petri dishes by spread plate method thus was allowed to set firmly for (5) minutes after ward inoculated plates were incubated at 37°C for 24 hours.

The collected swabs were submitted to culture by inoculation into nutrient broth and incubated at 37°C for 5 hr. (enrichment step) to increase bacterial level according to.(Stormberg, *et al.,* 2015). Loop full from the incubated broth was distributed onto surface of MacConkey agar then incubated at 37°C for 24 hr.

Identification of bacterial species:

Bacterial identification was done by biochemical test and by vitek 2

The growing colonies transferred to new specialized media for each bacteria to obtain for a pure culture. Again the isolated bacteria were cultured at a 37^{0}C for 24 h and staining procedure were applied by using Gram stain. The biochemical tests were conducted by Vitek 2, Kit to identify the isolated bacterial species.

Gram Stain: according to (McFadden, 2000).

Statistical analysis

Statistical analysis of data was performed using ANOVA, unpaired t test and lowest significant differences (LSD). All experimental data are presented as Mean ± S.E. The results were considered significant of $P < 0.05$. (Steel *et al.,* 2007).

Results

Workers and their equipment:

Workers

From total 150 swabs obtained from workers; only 12 (8.0%) samples were free from bacteria. While from 138 (92.0%), 149 isolates were isolated, either in single form (127)) [*Kleb*. 33; *Staph*. 24; *E. coli* 23; *Pseud*. 18; *Proteus* 9; *Yersinia* 8; *Streptococcus* 6; *Citrobacter* 2; *Enterobacter* 2, *Listeria* 1 and *Shigella* 1], or mixed, in two isolates in a sample (11); The highest isolate was, *Kleb*. 37/149 (24.8%), *Staph*. 32/149 (21.5%); *E. coli* 27/149 (18.1%), *Pseud*. 18/149 (12.1%); *Proteus* 9/149 (6.0%), *Yersinia* 8/149 (5.4%), *Streptococcus* 6/149 (4.0%), *Enterobacter* and *Citrobacter* each, 4/149 (2.7%), List. 3/149 (2.0%) and *Shigella* 1/149 (0.7%) table 1.

Table 1: Isolates from worker and their equipment.

Origin	No.	Isolates sp.												Total
		Kl.	Ps.	Sta.	Esch	Sal.	List.	Pro.	Citr.	Ent.	Str.	Sh.	Yers.	
Worker	150	37	18	32	27	0	3	9	4	4	6	1	8	149
Equip.														
Shop.	6	2	2	2	2	0	0	2	1	1				12
Chick.	7	1	1	3	2	2	0	2	1	1				13
Restau.	12	4	5	5	3	2	0	1	2	1				23
Equip.	25	7	8	10	7	4	0	5	4	3				48
worker	150	37	18	32	27	0	3	9	4	4	6	1	8	149
Total	175	44	26	42	34	4	3	14	8	7	6	1	8	197

Worker's equipment

From a total 25 swabs (6 from butchers shops; 7 from equipment used in poultry shops and 12 used in restaurants). From these 5 (20.0%) samples were free from contamination; from others 20 (80%), 48 isolates were isolated, either in single form (Sal. 1), two isolates (10), or three isolates (9). Highest one staph. 10/48 (20.8%); Pseud. 8/48 (16.7%); Kleb. and *E. coli* 7/48 each (14.6%); Prot.5/48 (10.4%), Sal. and Cit. each 4/48 (8.3%); Ent. 3/48 (6.3%) table 1.

From 175 samples collected from workers and their equipment. 17 (9.7%) did not yield bacterial isolates; while from others, 158 (90.3%), 197 isolates were isolated; either in single isolates (128)) [Kleb, 33; Staph. 24; *E. coli*, 23; Pseud.18; Proteus, 9; Yersinia, 8; Streptococcus, 6; Citrobacter, 2; Enterobacter, 2, listeria, 1, Sal. 1 and Shigella, 1]; or mixed in two isolates (21), or three (9). The highest isolate was Kleb.44/197 (22.3%); followed by Staph. 42/197 (21.3%); *E. coli* 34/197 (17.3%); Pseud. 26/197 (13.2%); Prot. 14/197(7.1%); Yersinia and Cit. each 8/197(4.1%); Ent. 7/197 (3.6%); Strept. 6/197(3.0%); Sal. 4/197 (2.0%); list. 3/197 (1.5%); Shigella, 1/197 (0.5%) table 1.

Sheep's raw meat

From 10 sheep's raw meat samples, Only 1 (10%) was free from bacterial contamination; while from others, 9 (90%), 16 isolates were isolated, either in single form (3) [Pseud. 2 and Kleb. 1], or mixed in two isolates (5), or three isolates (1) in a sample. The highest number of isolate was *Staph.* sp., 5/16 (31.2%); *Pseud.* sp., 3/16 (18.8%); Kleb.*; E. coli* Sal. and List. each of 2/16 (12.5%). table 2.

Sheep's meat products

From 5 sheep's meat products, only 1 (20.0%) was free from contamination, from other 4 (80.0%) , 9 isolates were isolated as mixed; either in two isolates (3), or three isolates (1). The highest number was Staph.3/9 (33.33%), followed by Kleb. and pseud. each 2/9 (22.22%); *E. coli* and List each of 1/9 (11.11%). table 2.

Beef

From 12 beef samples, only 2 (16.7%) were free from contamination, from other 10 (83.3%), 19 isolates were isolated, either in single (3) [Sal. 1 , *E. coli*. 1 and Staph. 1], or in two isolates (5) or three isolates (2) in a sample. The highest one was Staph 7/19 (36.8%), Kleb. 5/19 (26.3%), Pseud 3/19 (15.8%), List 2/19 (10.5%), *E. coli* and Sal. each 1/19 (5.3%). table 2.

Beef products

From 8 beef products, only 2(25.0%) were free from contamination. From others 6 (75.0%), 12 isolates were isolated. One isolate [List.1], Two isolates (4) and three isolate (1). The highest number were Kleb. and Staph. each 4/12 (33.4%); Pseud. List.; Cit and Ent. each of 1/12 (8.3%). table 2.

Poultry's raw meat

Table 2: Isolates from raw meat (sheep and calf).

Origin	No.	Isolates sp.									Total
		Kl.	Ps.	Sta.	Esch	Sal.	List.	Pro.	Citr.	Ent.	
Sheep											
Raw	10	2	3	5	2	2	2	0	0	0	16
Prod.	5	2	2	3	1	0	1		0	0	9
Total	15	4	5	8	3	2	3	0	0	0	25
Calf											
Raw	12	5	3	7	1	1	2	-	0	0	19
Prod.	8	4	1	4	0	0	1	-	1	1	12
Total	20	9	4	11	1	1	3	-	1	1	31
Raw											
Sheep	10	2	3	5	2	2	2	0	0	0	16
Calf	12	5	3	7	1	1	2	-	0	0	19
Total	22	7	6	12	3	3	4	0	0	0	35
Prod.											
Sheep	5	2	2	3	1	0	1		0	0	9
Calf	8	4	1	4	0	0	1	-	1	1	12
Total	13	6	3	7	1	0	2	-	1	1	21

From 26 poultry's meat, only 2 (7.7%) were free from contamination, while from others 24 (92.3%) samples; 50 isolates were isolated, either in single form [*E. coli* 1], or mixed, in two isolates in a sample (17), or three isolates (5). High isolate was Staph. 10/50 (20.0%); Sal. 9/50 (18.0%); *E. coli* 8/50 (16.0%); Pseud. 6/50 (12.0%); List. and Cit. 5/50 (10.0%); Kl. 4/50 (8.0%); Ent.3/50 (6.0%) table 3.

Poultry's meat products

From a total of 15 poultry's meat product, 2 (13.3%) were free from contamination, while from other 13(86.7%), 25 isolates were isolated, either in single form (5) [Ent. 1, Sal. 1, Staph.1, Kleb. 1, and *E. coli*. 1.], or in two isolates (4), or three isolates (4). Staph. 6/25 (24.0%); Kleb. 5/25 (20%) ; *E. coli* 4/25 (16.0%); Pseud. and Sal. 3/25 (12.0%); List.; Ent. each 2/25 (8.0%) table 3.

Poultry's meat and meat products

From 41 poultry's meat and meaty products, 4 (9.8%) were free from contamination, from others 37 (90.2%), 75 isolates were isolated, either in single form (6), or mixed , in two isolates (21), or three (9) isolates in a sample. Staph 16/75 (21.3%); Sal. and *E. coli* 12/75 (16.0%); Kleb. and Pseud. 9/75 (12.0%);List. 7/75 (9.3%); Cit. and Ent. 5/75 (6.7%) table 3.

Total isolates

From 251 samples, (328) isolates were isolated. The highest isolates was *Staphylococcus* sp. 77/328 (23.5%); followed by Klebsiela. sp. 66/328 (20.1%); *E. coli* 50/328 (15.2%), *Pseudomonas* sp. 44/328 (13.4%); *Salmonella* sp. 19/328 (5.8%); *Listeria* sp.16 / 328 (4.9%); Proteus and Citrobacter each 14/328 (4.3%), Enterobacter 13/328(4.0%), *Yersinia* sp., 8/328 (2.4%); Streptococcus 6/328 (1.8%) and *Shigella* sp. 1/328 (0.3%) table 4.

The isolates were either in single 141 out of 328 (43.0%) or mixed 187 (57.0%) with one or more other isolates table 5.

Klebsiella spp., was the highest isolates from workers, and their equipment (44/175 (25.1%), then from sheep and cow 13/35 (37.1%) and poultry 9/41 (22.0%). *Staph.* sp. 42/175 (24.0%), from sheep and cow 19/35 (54.3%), then from poultry 16/41(39.0%). While *Pseudomonas* sp.,26/175, 9/35 and 9/41 and *E. coli* 343/175, 4/35, 12/41, Sal, 4/175, 3/35, 12/41, List. 3/175, 6/35, 7/41, Prot. 14/175, Citr, 8/1765, 1/35, 5/41, Ent. 7/175, 1/35 , 5/41, Strep. 6/175, Yersinia 8/175, Shig1/175 table 4.

The isolates were either in single 141 out of 328 (43.0%) or mixed 187 (57.0%) with one or more other isolates table 6.

Table 3: Total numbers of isolates from sheep and calf meat and meat products.

Origin	No.	Isolates sp.									Total
		Kl.	Ps.	Sta.	Esch	Sal.	List.	Pro.	Citr.	Ent.	
Raw	22	7	6	12	3	3	4	-	0	0	35
product	13	6	3	7	1	0	2	-	1	1	21
Total	35	13	9	19	4	3	6	-	1	1	56

Table 4: Isolates from poultry meat and meaty products.

Origin	No.	Isolates sp.									Total
		Kl.	Ps.	Sta.	Esch	Sal.	List.	Pro.	Citr.	Ent.	
Raw											
Chic.	20	4	5	7	7	6	2	-	3	2	36
Turk.	6	0	1	3	1	3	3	-	2	1	14
Total	26	4	6	10	8	9	5	-	5	3	50
Prod.											
Total	15	5	3	6	4	3	2	-	0	2	25
Chicken	20	4	5	7	7	6	2	-	3	2	36
Turkey	6	0	1	3	1	3	3	-	2	1	14
product	15	5	3	6	4	3	2	-	0	2	25
Total	41	9	9	16	12	12	7	-	5	5	75

Discussion

Meat and meat products contamination

The results of current study showed that 27 out of 251 (10.8%) of samples depended in study did not yield isolates. While, 224 (89.2%) yield isolates.

Ladaporn *et al.,* (2013). found that only, 3% of raw food samples were found to be free of bacterial enteric pathogens. (Shahram *et al*., 2012). Found that culture samples of the 70 (52.3%) out of 134 sandwiches from markets and supermarkets in Kerman city, Iran were positive, yielding 4 bacteria, one fungi and 64 samples (47.7%) had no bacterial growth.

Table 5: Total isolates which were isolated in current study.

Origin	No.	Isolates sp.												Total
		Kl.	Ps.	Sta	E	Sal	List	Pro.	Citr.	Ent.	Str.	Yer.	Sh.	
Workers	175	44	26	42	34	4	3	14	8	7	6	8	1	197
Sheep, beef	35	13	9	19	4	3	6		1	1				56
poultry	41	9	9	16	12	12	7	0	5	5				75
Total	251	66	44	77	50	19	16	14	14	13	6	1	8	328

Table 6: Total numbers of isolates in current study.

Origin	No.	Species of isolates												Total
		Kl.	Ps.	Sta.	Esch	Sal.	List.	Pro.	Citr.	Ent.	Str.	Sh.	Yers.	
workers	150	37	18	32	27	0	3	9	4	4	6	1	8	149
Equip.	25	7	8	10	7	4	0	5	4	3				48
Sheep raw	10	2	2	4	1	0	2	0	0	0				11
Beef	12	5	3	7	1	1	2		0	0				19
Turkey meat	6	0	1	1	1	2	0	0	0	0				5
Sheep prod.	5	2	2	3	1	0	1		0	0				9
Beef prod	8	4	1	4	0	0	1		1	1				12
Chicken meat	20	2	3	4	5	4	1		1	1				21
Turkey liver	6	0	1	3	1	3	3		2	1				14
Chicken product.	15	5	3	6	4	3	2		0	2				25
Total	131	33	31	56	25	15	14		10	10				201
Total	251	66	44	77	50	19	16	14	14	13	6	1	8	328

The results revealed from 251 samples, (328) isolates were isolated. The common isolates were Staphylococcus sp. 77/328 (23.5%); followed by *Klebsiela.* sp. 66/328 (20.1%); *E. coli* 50/328 (15.2%), *Pseudomonas* sp. 44/328 (13.4%); *Salmonella* sp. 19/328 (5.8%); *Listeria* sp. 16/328 (4.9%); Proteus and Citrobacter each 14/328 (4.3%), Enterobacter 13/328(4.0%), *Yersinia* sp., 8/328 (2.4%); Streptococcus 6/328 (1.8%) and *Shigella* sp. 1/328 (0.3%). The results showed that 229 isolates out of total isolates 328 (69.82%) were belong to enterobactericeae.

The presence of bacteria in meat has been widely reported from different parts of the world (Holds, *et al.,* 2007; Kinsella *et al.,* 2008). The predominant bacteria isolated and identified from different sites of bovine carcasses were *E. coli* , *Salmonella* spp. and *S. aureus* (Das *et al.,* 2019). Overall the *S. aureus* (78.5%) was found predominant, followed by *E. coli* (64.0%) and *Salmonella* spp. (64.5%) and most of the carcasses were contaminated concurrently with all the three types of bacteria. The earlier reports on the contamination of Coliform, Staphylococci, Lactobacillus, Micrococcus, Streptococci, Pseudomonas, Enterobacter, Bacillus, Proteus and Salmonella bacteria in freshly slaughtered meat and town market meat in Bangladesh (Khatun *et al.,* 2003).

It has been reported that Gram negative bacteria accounts for approximately 69% of the cases of bacterial food- borne disease, with the coliform being the most frequently identified group on meat which include *Citrobacter freundii*, *Escherichia coli* and less frequently of the genera Klebsiella, Salmonella, *Vibrio cholera, Shigella sonnie* and *Proteus* spp. (Clarence *et al.,* 2009). The most common appearance of coliform bacteria in meat samples was *E. coli* 34.3%, *Citrobacter* spp., 20%, Proteus spp. 14.3% and Enterobacter spp.14.3%.(Mansour,Abdalla Mohammed *et al.,* 2019).

Collins and Thato, 2011. Found that from 10 raw mincemeat collected retail shops in the South Africa, A total of 150 isolates. The proportion of enteric bacteria was higher in samples obtained from butchers (86.7%) than in the supermarkets (43.3%, 53.3%). The most frequently identified species were Serratia *odorifera* (17.3%), *E. coli* (10.0-%), Klebsiella *oxytoca* (6.7%) and Enterobacter *aerogenes* (6.0%). Enteric bacteria species were isolated and positively identified in all meat sample.

In current study, from 15 sheep's meat and meat products; 2 (13.3%) were free from contamination; while from other 13 (66.7%), 25 isolates were isolated either in single form (3); or mixed in two (8) or three (2). The Staph. was isolated in highest numbers 8/25 (32.0%); pseud 5/25 (20.0%); Kleb. 4/25 (16.0%); List. and *E. coli* 3/25 (12.0%); Sal. 2/25 (8.0%).

Bacterial colonies were isolated from all raw and finished mutton samples. A maximum of 41 colonies were observed in Raw Meat sample followed by 31 colonies in minced Mutton sample. For the Finished Product samples, number of colonies per plate ranged from 7 in mutton nuggets to a maximum of 58 colonies in Mutton dried Kebab sample. The common colonies were found to be *E.coli*, *Staphylococcus* sps, *Pseudomonas* sp., *Micrococcus* sp., *Streptococcus* sp., Serratia sp., *Shigella* sp., and *Salmonella* sp. in raw meat samples. Where as in Finished meat products *Salmonella* sp., *E.coli*, *Streptococcus* sp., *Serratia* sp., *Campylobacter* sp., *Proteus* sp. and *Klebsiella* sp. It is observed that the bioload was more in finished product (Dry Mutton Kebab) than the raw meat samples. This could be because of bacterial contamination from air as the samples were

kept outside in open area, (Syed and Sarangi, 2013).

From 20 beef and beef products, 4 (20.0%) were free from contamination; from other 16 (80.0%), 31 isolates were isolated, either in single isolate (4); two isolates (9); and three isolates (3) in a sample. The highest number of isolates was Staph. 11/31 (35.5%); Kleb. 9/31 (29.0%); Pseud. 4/31 (12.9%); List 3/31 (9.8%); *E. coli*; Sal.; Cit. and Ent. 1/31 (3.2%).

In current study, *E. coli* occupied the fourth position post Kleb., Pseud. and Staph. [50/351 (19.9%)], as it was isolated from poultry meat and meat products [12/41 (29.3%)]; from workers and their equipment [34/175 (19.4%)]; from sheep and cow [4/35 (11.4%)].

The incidence of *E. coli*, found by, (Zaki-Eman, 2003). (40%), While by, (Abou-Hassien-Reham, 2004). Founded (12%). Shahram *et al*., (2012). Found that sandwich samples, were heavily contaminated with *E. coli* spp. (40.3%), was the most prevalent food- borne pathogens isolate from Sandwiches, followed by *Staphylococcus aureus* (4.5%). Thirty four out of 56 Kaalbas sandwich samples (63%) showed bacterial contamination followed by hamburger (52.4%) and sausage (36.8%). (Shaltout, *et al.,* 2016; Ouf-Jehan, 2001). Found the incidence of *E. coli* (25%) in sausages. Presence of *E. coli* in meat indicates a general lack of cleanness during slaughtering, evisceration, dressing, transportation and handling of meat, (ICMSF, 1996).

Jar Allah *et al.,* 2014. demonstrated that meat samples from butchers shops in Kut City, were heavily contaminated with *E. coli* (40%), high contamination level of Coliforms in examined meat products may indicates unsanitary conditions of raw meat production from which produced. They are indicators of fecal pollution at slaughterhouse which begin from skinning and direct contact with knives and workers hands. Also, during evisceration and washing, contamination may come from intestinal content as well as from water during rinsing and washing of carcasses. This result was remarkably different from those reported by (Vazgecer *et al.,* 2004). (31%).

In current study Salmonella occupied the fifth position 19/251 (7.6%). It was isolated from poultry meat and meat product [12/41 (29.3%)], from sheep and cow's meat and meat products 3/35 (8.6%), from workers and equipment [4/175(2.3%)].

Salmonella spp. was isolated from 4(3%) of samples which is significantly lower than other reports (Little, *et al.,* 2007, Meldrum and Smitrh, 2007, Harakeh *et al.,* 2005; Kubota *et al.,* 2008, Toyofuku *et al.,* 2008, Van *et al.,* 2007). In minced meat, (Mohamed, 2013), found Salmonella in (40%), while (Stock and Stolle, 2001). Found it in low level (15.8%), (Fritzen *et al.,* (2006). Found it in higher levels (69.5%). (Forsythe 2000). *Salmonella* spp. are poorly isolate from beef and sheep meat while it is easy to isolate from poultry meat. Chicken meat consider main sources of infection for human and animals. The identified *Salmonella* spp. isolates by (Hassnien - Fatin (2004). in (12%). While (Mremas - Neema *et al.,* (2006). in (26%) of sausage. Meanwhile (Sharma *et al.,* (2002). Found Salmonella in (3.23% of Kofta).

In current study, from 20 beef and beef products, 4 (20.0%) were free from contamination; from other 16 (80.0%), 31 isolates were isolated, either in single isolate (4); two isolates (9); and three isolates (3) in a sample. The highest number of isolates was Staph. 11/31 (35.5%); Kleb. 9/31 (29.0%); Pseud. 4/31 (12.9%); List 3/31 (9.8%); *E. coli*; Sal.; Cit. and Ent. 1/31 (3.2%).

Nura and Yousef *et al.,* (2014). Found that beef burger samples were highly contaminated with several pathogenic bacteria such as *E. coli* (74.5%), *E. coli* O157:H7 (27.1%), *S. aureus* (28.8%) and Aeromonas (18.6%). Meanwhile, fresh sausages samples were contaminated with *E. coli* O157:H7 (39.3%) and Salmonella (2.1%). In addition, studies on chicken burgers, found that % of *E. coli* and *E. coli* O157:H7 reached (10.9%) and (4.68%) respectively.

Jarullah, (2015), referred that from 30 samples of meat (10 beef meat, 10 from sheep and 10 from poultry meat), *E. coli*, Klebsiella, *Staphylococcus aureus*, Shigella, *Salmonella* spp., *Bacillus cereus*, Enterobacter, Proteus, Micrococcus, *Pseudomonas* spp. and *Streptococcus* spp. were isolated. The predominant bacteria was *E. coli*, about 35% in all samples, followed by *Staphylococcus* spp. 25%.

In Tripoli, study found that 74.5% of meat samples were contaminated with *E. coli*, 18.6% Aeromonas, 28.8% *Staph. aureus*, 27.1% *E. coli* H7:O157 and 2.1% Salmonella (Altajori and Elshrek 2014). While, (El Shrek and Ali (2012). In Tripoli restaurants, found 25.9% Proteus, 29.6% *Staphylococcus aureus*, 20.3% *E. coli*, H7: O157 and 12.9% in Salmonella.

In one Iraqi study that compared imported meat to local meat, demonstrated that predominant bacteria were *E. coli* then *Staphylococcus* spp. also the count of bacterial contamination near the standard Iraqi values. No isolates of Salmonella from local and imported beef and sheep meat (Samir, *et al.,* 2013).

Staphylococcus

Staph. sp. occupied the third position in current study

(77/251(30.7%), as it was isolated from sheep and cow's meat and meat products [(19/35(54.3%)], from poultry meat and meat products [(16/41(39.0%)] and from workers and equipment 42/175(24.0%).

S. aureus frequently are present in low numbers on raw meat surface occurs infrequently (Saadia and Hassanein, 2010; Jaralah, 2014). demonstrated that fast foods samples from restaurants in Kut city were heavily contaminated with *S. aureus* (28%). This represent a high level of contamination indicates a potential breakdown of hygiene at various stages of the food processing, *Staph. aureus* is most likely transmitted by hand of food workers (Todd *et al.,* 2008).

In current study, Listeria occupied the sixth position (16/251(6.4%). As it was isolated From sheep and cow's meat and meaty products (6/35(17.1%)] from poultry meat and meaty products [7/41(17.1%); and from workers and equipment [3/175(1.7%)].

Listeria species were isolated from 25 (22.94%) out of 109 samples of fresh and processed meats. sixteen of 50 beef, 1 of 7 pork samples and 8 of 52 sausages samples were positive for listeria (Doijad *et al.,* 2010). Similarly Listeria spp. has been isolated from 25% and 29% of samples from local and internet market, respectively in USA (Pao and Ettinger, 2009). About 5% samples of raw beef were contaminated with *L. monocytogenes*. High prevalence of Listeria species was also recorded in beef in Ethiopia (Mengesha *et al.,* 2009).

In current study, from 41 poultry's meat and meaty products, 4 (9.8%) were free from contamination, from others 37 (90.2%), 75 isolates were isolated, either in single form (6), or mixed , in two isolates (21), or three (9) isolates in a sample. Staph 16/75 (21.3%); Sal. and *E. coli* 12/75 (16.0%); Kleb. and Pseud. 9/75 (12.0%); List. 7/75 (9.3%); Cit. and Ent. 5/75 (6.7%).

Poultry meat is found contaminated with *E. coli*, *Salmonella* spp. and *Staphylococcus* spp. (Malmuthuge *et al.,* 2012; Sudershan *et al.,* 2012; Voidarou *et al.,* 2011; Torok *et al.,* 2011; Petrovic *et al.,* 2011; Awad-Alla *et al.,* 2010; Ahmed *et al.,* 2009). In studies in Bangladesh they reported the presence of *E. coli*, *Salmonella* spp., *Staphylococcus aureus* in poultry meat and chicken rinse samples.

Shah *et al.,* 2012). reported that the high prevalence of Salmonella in chicken meat may be due to cross-contamination from intestines during processing and cutting or from cages, floor and workers during retailing or marketing. They also concluded that water used for washing of carcasses may be responsible for this and the meat could be contaminated with Salmonella from feces or from the butcher's hands during washing (Shah *et al.,* 2012).

High prevalence of Salmonella spp. Isolated from broiler meat by (Noori and Alwan, 2016), may indicated that the broiler carcasses may contaminated with Salmonella during slaughtering process or evisceration, these idea was agreement with (Sakaridius *et al.,* 2011). Who explained that the contamination of poultry meat by Salmonella serotypes can occur through slaughtering and evisceration process.

Five bacterial serotypes were isolated from broiler meat including 39 *Salmonella* spp., 6 *Pseudomonas* spp., 6 *Citrobacter*, 29 *E. coli* and 5 Proteus. Most of these isolates were pathogenic for human, these result indicated that broiler meat is important source of food borne pathogen that may be cause human illness or dead if there is not good treatment, these idea was in consistent with (Paterson, 2006). Who showed that beef and chicken meat contaminated with fecal organisms may considered essential food hygiene problem particularly Enterobacteriaceae including *Salmonella* spp., *E. coli, Proteus* as well as *Klebsiella* spp (Paterson, 2006; Zahao and Doyle, 2001). High mortality (2.2 million individual dead) in human population was occur by food and water borne diarrheal disease every year (FAO, 2006).

Isolation of *L. monocytogenes* from chicken meat was recorded previously by many investigators (Miettinen *et al.,* 2001; Akpolat *et al.,* 2004; Lekroengsin *et al.,* 2007; El-Shabacy Rasha, 2008; Keeratipibul and Lekroengsin, 2009; Ahmed and El- Atti, 2010). A total of 95(38%) suspected *Listeria* sp., were isolated from 250 samples of retail broiler chicken. Frozen chicken burger 22 isolates (44%) were detected, frozen chicken sausages 11 isolates (22%), Frozen chicken meat balls (Kofta) 27 isolates (54%), chicken shawerma 26 isolates (52%) and chicken mortedella 9 isolates (18%). (Alsheiukh *et al.,* 2013). The presence of *L. monocytogenes* in processed meats could be explained by the inadequate heat treatment to destroy the growth of *L. monocytogenes* or as a result of post process contamination.

Staphylococcus sp. was isolated in highest numbers from samples obtained from sheep and cow's meat and meat products [19/35 (54.3%)], then from poultry meat and meat products [16/41(39.0%)] and lastly from workers and equipment [42/175 (24.0%)]. *Klebsiella.* Sp., was isolated in highest number from sheep and cow's meat and meat products [13/35 (37.1%)], from workers and equipment [44/175 (25.1%)]. From poultry meat and meat products [9/41(22.0%)] . While *Pseudomonas* sp.,

was isolated in higher number from sheep and cow's meat and meat products [9/35(25.7%),%]; then from poultry meat and meat products [9/41(22.0%)] and from worker and equipment [(26/175(14.9%)]. *E. coli* from poultry meat and meat products [12/41(29.3%)]. Workers and equipment [34/175(19.4%)], from sheep and cow's meat and meat products [4/35(11.4%)], Salmonella were isolated in higher number from poultry meat and meat products {12/41(29.3%)], then from sheep and cow's meat and meat products [3/35(8.6%)], from workers and equipment [4/175(2.3%)]. *Listeria* sp. from poultry meat and meat products [7/41(17.1%)], from sheep and cow's meat and meat products [6/35(17.1%)] and from workers and equipment [3/175(1.7%)]. Citrobacter from poultry meat and meat products [5/41(12.2%)], from workers and equipment [8/175(4.6%)] and from sheep and cow's meat and meat product [1/35(2.9%)]. Enterobacter from poultry meat and meat product [5/41(12.2%)] from workers and equipment [7/175(4.0%)] and from sheep and cow's meat and meat products [1/35(2.9%)]. *Proteus* sp. only isolated from workers and equipment [14/175(8.0%)]. *Yersinia* sp. 8/175(4.6%)]; *Streptococcus* sp. only from workers and equipment [6/175(3.4%)] and *Shigella* sp. [1/175(0.6%)].

The results of current study, revealed from 251 samples, (328) isolates were isolated. The common isolates were *Staphylococcus* sp. 77/328 (23.5%); followed by *Klebsiela.* sp. 66/328 (20.1%); *E. coli* 50/328 (15.2%), *Pseudomonas* sp. 44/328 (13.4%); *Salmonella* sp. 19/328 (5.8%); *Listeria* sp.16 / 328 (4.9%) ; Proteus and Citrobacter each 14/328 (4.3%), Enterobacter 13/328(4.0%), *Yersinia* sp., 8/328 (2.4%); Streptococcus 6/328 (1.8%) and *Shigella* sp. 1/328 (0.3%). The results showed that 229 isolates out of total isolates 328 (69.82%) were belong to enterobactericeae.

The current study allows to conclude that the possibility of contamination of meat products with such serious pathogens remains as a public health problem. Thus all precautions of proper sanitation during manufacture, handling and storage of such meat products should be adopted to control these serious pathogens and to obtain a maximum limit of safety to consumers.

In recent years, Iraqi local markets were invaded by various food stuff from different known and unknown origins regardless whether such food valid for human consumption or not. Also, the lack of proper requirements of transporting, storing and marketing of such food may result in contamination of the food with various physical, chemical, and biological contaminants that may form serious health threats particularly of those imported products. The refrigerated poultry meat would be spoilage when stored for a long period due to the microorganism actions in addition to the biochemical transformations inside the product (Octavian, *et al.,* 2010; Al-jasser, 2012).

In Iraq, poultry slaughtered manually, therefor, contaminated by different types of microorganisms bacteria, fungus even parasites from soil or from contaminated earth with other poultry wastes (Anjum, *et al.,* 2004; Siddiqui, *et al.,* 2008). Sometimes the washing is unbeneficial to be pure from microorganisms and we need immediately cooking for meat to kill these creatures especially pathogenic one (Siddiqui, *et al.,* 2006), While the frozen poultry meat which comes to markets from unknown origins may be contaminated by certain microbial pollutants, because unknown sources, processing and transporting in addition to instability of electrical power.

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