



Isolation and Identification of Zoonotic Bacteria from Poultry Meat

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Abstract

In order to determine the prevalence of Zoonosis bacteria in the local and imported broiler meat in local markets of Baghdad city, one hundred of frozen broiler meat were collected from local markets. Bacteria isolated were done by routine cultivation and identification by biochemical test and API₂₀ E system. The result showed that among 100 broiler meat samples, 85% were bacterial positive isolates and in local broiler meats, 37 out of 49 were positive isolated while in imported broiler meat, 48 out of 51 were bacterial positive isolates. Among 48 local broiler meat, it was reported 39% *Salmonella* spp, *E.coli* 29% , 6% *Pseudomonas* spp, 6% *Citrobacter* and 5% *Protous* spp. The present study showed that the main *Salmonella* spp isolates are *S.infantis* 0.54%, *S.vichow* 0.13%, *S.enteritidis* 0.21%, *S.hato* 0.08%, *S.dublin* 0.05%. It was recorded that *Salmonella infantis* was high resistant to intermediate resistant to Ciproflaoxacin (CIP10) Amikacine (AK10) Gentamicin (CN10). It was conclusion that imported broiler meat were considered important source of Zoonosis food borne bacteria particularly multi antibiotic resistant *S.infantis*.

Keywords: Zoonosis bacteria, frozen broiler meat, multi antibiotic resistant.

Introduction

The main public health and economic problem is food borne disease which increase worldwide particularly in individuals eat meal outside their home, due to uncontrolled hygienic preparation of these type of the food, food borne disease was define according to WHO, infectious ortoxic nature of the disease occur through consumption of contaminated food (le Loir et al 2003). Food borne diseases(FBD) were affected one third population every year world word. In the developing countries and developed countries such as in USA in which billions of dollars were spent in the treatment of food borne pathogens that affected approximately 48millions patients each year (Scallan et al., 2011). However, the main source of food borne disease are the animals (Busani et al 2006), these disease cause huge public health and economic problems (Gajadhar and Allen 2004), high

mortality (2.2 million individual dead)in human population was occur by food and water borne diarrheal disease every year (FAO/WHO 2006).

Due to rapidly increasing in human population and changing in urbanization food habits, increasing in animal products consumption such as meats were recorded, at year 2030,FAO suspected consumption of meats and milk products would significantly increase associated with mass production and movement meat products, globally in meats production(FAO,2006), these condition may lead to give a good chance for food contamination and spread food borne pathogens that animal origin which including *Sallmonella* spp, *Campylobacter*, *E.coli*, *Staphylococcus* spp, *Cloxytidium*, *Yersinia*, *Listeria*, *Acrobacter*, *Mycobacterium*, *Trichinella*, *Sarcocystis*,

Toxoplasma gondii and *Cryptosporidium parvum* (Dhama et al., 2013).

Beef and chicken meat contaminated with fecal organisms may be considered essential food hygiene problem particularly Enterobacteriaceae including *Salmonella* spp, *E.coli*, *Proteus* as well as *Klebsiella* spp (Paterson 2006). Malpractices handling of poultry meat lead to food contamination with food borne microbes, in addition poultry may be considered important food borne pathogen reservoir such as *Salmonella* spp and *Campylobacter enteritis* as a result of these organisms appear asymptomatic in live birds as well as large number of bird carcasses remain together during the operation and processing methods (Cavitt, 2003). Widely using antibiotic in the poultry as treatment prophylactic or growth promoters in live stock lead to widely spread antibiotic resistant pathogens that cause problem in humans particularly Salmonellosis (Schroeder et al., 2004). Threlfall et al., 2003 found that 40 percentage of *Salmonella typhimurium* isolated from humans expressed single or multidrug resistant in certain European countries in 2000, also Chung et al., 2004 isolated antibiotic resistant pathogen from poultry meats particularly *Salmonella* and *E.coli*. Nadeau et al., 2002 recorded that bad handling and consumption undercooked meat of poultry were the main source of meats contamination with *Salmonella* and *Campylobacter*, these two organisms were associated with raw chicken (Hernandez et al., 2005). Also these organisms were isolated from food and water of poultry (Padungtod and Kaneene, 2005). Majority of poultry meats were come to Iraq from different sources particularly from India, Iran and China, meat of chicken are stored in freezers but there is not constant power of electric supply in the local markets which are using fuel powered generating sets, therefore these meats may be exposed to growth contaminated pathogens which may originate from contaminated chicken carcasses by their gut containing such as *Salmonella* and *E.coli* which were considered a main food borne pathogen (Adesiji et al. 2011). In Iraq, there is little information about the zoonosis bacteria in broiler meats, therefore

The aims of the present study were:

1. Determine the prevalence of bacterial isolates in imported and local broiler meat
2. Identify the *Salmonella* serotype isolates
3. Determine the sensitivity of *S.infantis* to antibiotic drugs

Materials and Methods

1. Sample collecting

The collection of samples was done during the period from Oct. 2015 until Feb. 2016. For achieving the aims of this study, 100 raw and freezing broiler carcasses were randomly collected from different markets at Baghdad city in Iraq. The samples were directly transferred in an icebox to the laboratory for further preparation and examination.

2. Bacterial isolation

100 samples of poultry meat were collected from local markets. The procedure has been done by putting 25 gm of meat in 225 ml of nutrient broth overnight at 37°C then taking 1ml from the broth put in 10 ml of peptone water by making serial dilution from 1:10 – 1:1000 and 1 ml put on MacConkey agar which incubation at 37°C overnight.

At the same time, it was taken 1ml from 225 nutrient broth that was incubated overnight and put it in 9ml of Tetrathionate broth + Brilliant green and incubated overnight for *Salmonella* ssp. The growth then cultured onto XLD, HE, Bismuth and SS agar.

3. Bacterial identification

Bacterial identification was done by biochemical test and by API 20.

4. Serological test

After diagnosis of each isolate serotyping of each one was done at Central Laboratories of Ministry of Health in Baghdad.

5. Sensitivity test

S.infantis isolates were tested for their antimicrobial resistance/susceptibility pattern by disc diffusion technique according to Clinical and Laboratory Standards Institute (CLSI, 2000). This test was done by using Kirby-Bauer disk diffusion test

Results

The Results of isolation and identification. The bacteriological culturing expressed different shape, color and consistency of colonies on XLD, gram stain showed some of these culturing MO were negative and other positive gram stain, by using selective media such as XLD, SS, HE and bismuth agar,

the colonies appear Black color (fig:1,2), also gram stain expressed gram negative rod shape MO, the bacterial isolates were confirm diagnosis by biochemical test (Table:10) and by Api test, these tests revealed different bacteria species that isolated from broiler meat.

The result showed that among 49 local and 51 imported broiler meat samples, 37 (13.69%), and 48 (23.4%) respectively were positive for bacterial isolates (Table :1), also the result expressed that among 37 local broiler meat, the bacterial isolates from broiler meat collected from local markets including *Salmonella* spp,19, *Pseudomonas* spp 2, *Citrobacter* 4, *E.coli* 10 and *Proteus* ssp2 (Table:2).

39 spp isolated from frozen meat which including *S.infantis* 21(0.54%), *S.vichow* 5 (0.13%), *S.enteritidis* 8(0.21%), *S.hato* 3(0.08%) and *S.dublin* 2(0.05%) (Table: 3). The results of antibiotic resistant were determined by compared the diameter of the zones of complete inhibition with the zone size interpretation chart provided by the supplier and was graded as susceptible (S), intermediate (I), and resistant (R), the present result revealed that *S.infantis* highly (100%) resistant to Piperacillin (PRL30) and to Cefotaxime (CTX10), and sensitive resistance to Ciproflaxacin (CIP10), Amikacine (AK10) and Gentamicin (Table:4).

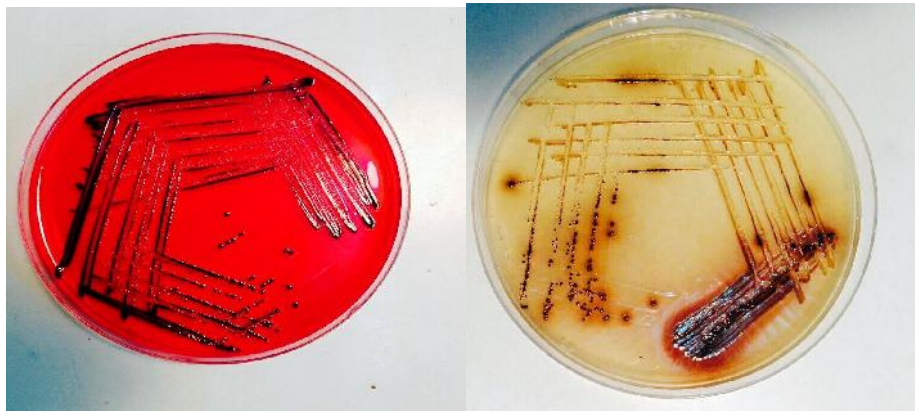


Fig1: shows in the right black colonies on XLD agar & Browne colonies on Bismouth agar in the left

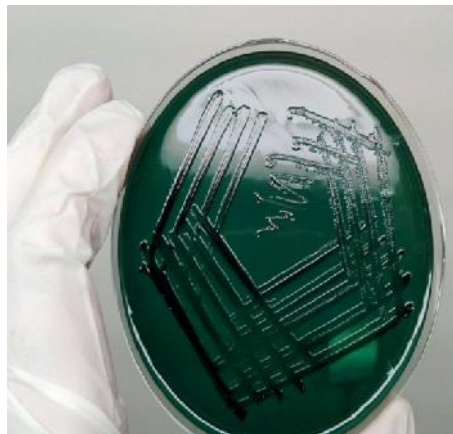


Fig 2: shows green colonies on HE agar



Fig3: shows *S.infantis* on Gram negative stain pink color rode shape

Table1: shows number and percentage of bacterial isolation according to source of broilers.

Type of specimen	No. of specimen	Positive isolates	Percentage of Positive isolates
Local broilers	49	37	13.69%
Imported broilers	51	48	23.04%
Total	100	85	72.25%

Table 2 : Shows bacterial species isolated from the importer and local broilers.

Type of broilers	No of +ve specimen	Salmonella ssp	E.coli	Proteus ssp.	Citrobacter ssp.	Pseudomonas ssp.
Local	37	19	10	2	4	2
Imported	48	20	19	3	2	4
Total	85	39	29	5	6	6

Table: 3: showed total number of Salmonella spp and strain and their number of Salmonella species isolated from the broiler.

Salmonella ssp	<i>S.infantis</i>	<i>S.vichow</i>	<i>S.enteritidis</i>	<i>S.hato</i>	<i>S.dublin</i>
No.	21	5	8	3	2
percentage	0.54%	0.13%	0.21%	0.08%	0.05%

Table 4: shows sensitivity of *Salmonella infantis* to certain antibiotic.

Type of antibiotic	resistance	intermediates	sensitive
piperacillin (PRL30)	+	-----	
Cefotaxime (CTX10)	+	-----	
Ciproflaoxacin (CIP10)		-----	+
Amikacine (AK10)		-----	+
Gentamicin (CN10)		-----	+

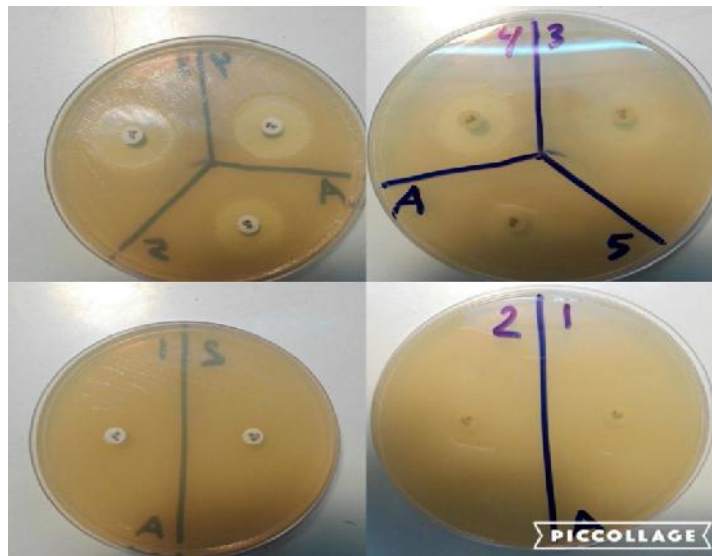


Fig 5: sensitivity test for *Salmonella* spp. The piperacillin and cefotaxim are resistant and the others are sensitive

Discussion

The current study showed highly prevalence of bacterial isolates in the raw and frozen chicken meat (85%) and the imported frozen chicken meat expressed 51(43.4%) of bacterial isolates as compared with local broiler meat 49(40.8%) these result may indicated that the poultry meat was considered a source of bacterial infection, particularly imported chicken meat, of humans after bad handling or consumption undercooked meat, specially the Iraqi people consumed large amount of poultry meat and the main source of these meat is imported frozen chicken meat which contaminated with different bacterial species during bad handling transmitted and storage in the markets due instable electric source, these idea was agreement with observation of Mor-Mur and Yuste, 2010.who reported that the poultry meat food can be contaminated with bacteria agents during different steps of food preparation including production, processing ,distributing, storage and retailing in the markets, however, poultry meat is considered a main source of meat world(Kearney, 2010).it required hygienic safety of the meat and meat products in order to prevention food borne disease transmission worldwide (Mor-Mur and Yuste, 2010) in which poultry and their products were considered essential vehicle for transmission of these disease (Tauxe and Blake, 1992).also the present finding was supported idea that poultry meat is important source of human infection ,these idea was agreement with 1\five who reported that the main source of food borne disease are the animals ,these disease cause huge public health and economic problems, animal origin food were the main source

of food borne disease which cause serious problem in both developing and developed countries particularly in the immunocompromised patients and malnutrition (Gajadhar and Allen 2004).

The current result demonstrated that 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 (Zahao 2001, Paterson 2006), high mortality (2.2 million individual dead) in human population was occur by food and water borne diarrheal disease every year (FAO 2006).

Gajadhar and Allen 2004 considered that animal food origin were the main source of food borne pathogens, The current (85%) was isolated from 100 frozen broiler meat ,it was determined that the meat poultry was considered unsafe when *Salmonella* present in more than 25 gram of poultry meat (Gladys and Olayinka), on the base of above result, we considered that broiler meat carried these zoonotic pathogen to the humans during bad handling or consumption undercooked of these meats, these result was agreement with Gladys and Olayinka, 2014 who reported that fifty three chicken meat that collected

from retail markets expressed 32.1% of *Salmonella* positive isolates also the current result was higher than those(11.1%) reported by (Ukut et al. 2010) in Calabar metropolis and by Adesiji et al.,2011(2 percentage) in Osogbo from Nigeria.

The prevalence of *Salmonella* isolates in the current study was varied from certain studies, these may be due to differences in sampling way, methods of diagnosis, season of initiation salmonellosis in livebirds as well and contamination during processing stages.

High prevalence of *Salmonella* spp isolated from broiler meat in the present study may indicated that the broiler carcasses may contaminated with *Salmonella* during slaughtering process or evisceration , these idea was agreement with (Sakaridis 2011) who explained that the contamination of poultry meat by *Salmonella* serotypes can occur through slaughtering and evisceration process .

The high prevalence of *Salmonella* spp isolated from broiler meat in the present study may indicated that the broiler meat is one important source of human infection by food borne Salmonellosis also the present result may indicated widely spread of *Salmonella* in retail meat markets that consider a risk of human infection with *Salmonella* through handling in households or consumed insufficiency cooked process (Saikia 2010).

these idea was agreement Conner ,2002who concluded that *Salmonella* is considered one of important animal original food borne pathogen that human can be infected by these pathogen through cross contamination or ingestion undercooked meat, the high prevalence of *Salmonella* spp in the current study may be responsible for most food poisoning hospitalization patients in Iraq, these evidence was agreement with CDC , 2011 who estimated that the *Salmonella* causes one million infection with 378 death every year in USA. also *Salmonella* spp cause numerous food borne disease outbreaks in different countries, it induced 39.2% among 2201 outbreaks in the European union in the 2007, most of them occur in the France (European Food Safety Authority and European Centre for Disease Prevention and Control, 2011) The current study revealed that high prevalence of *Salmonella* isolates, these result was supported idea that the *Salmonella* spp were considered important etiology of food poisoning in poultry meat, these result was in consistent with

Medeiros et al.,2011 found that the prevalence of *Salmonella* spp was 2.7% in Brazilian.

The source of *Salmonella* in the present study may be endogenous that he life broiler may be infected asymptotically with these pathogen and the *Salmonella* activation and proliferation under improper temperature of storage in the markets or exogenous through contamination of broiler carcasses through slathering. poultry meat were considered important source of *Salmonella* infection of human due to chicken meat may be contamination by these organism during different steps of slaughter, enervation, processing, distribution, handling and storage (Gladys. and Olayinka,2014).

The current study revealed five serotypes of *Salmonella* isolated from frozen broiler meat including *S.infantis* (21)0.54%, *S.vichow* (5)0.13%, *S.enteritidis* (8)0.21%, *S.hato* (3) 0.08%, *S.dublin* (2)0.05%.,these result may give idea that the carcasses of broiler may be contaminated by these organism which considered a main food borne pathogen, from GIT containing, these idea was agreed with Assèta et al.,2013 who isolated several serotypes of *Salmonella* from poultry fecal samples such as *S.vircho*, *Shatoand S.typhimurium*, these serotypes commonly cause sporadic case of infection or outbreak in the humans (CDC 2006) Result of current study showed highly prevalence of *S.infantis* isolated from frozen broiler meat, these result may indicated that these serotype is the common meat broiler borne Zoonosis disease in frozen meat in the local and imported markets in Baghdad city, the current result was in consistent with Hamid et al., 2015 who recorded five serotypes of *Salmonella* spp in 19.8% of chicken meat samples and among these serotypes identified, *S.infantis* was the predominant type (48.7%) followed by *S.enteritidis* 22.5%, *S.typhimurium* 12.6%,*S.Newport* 7.2 and *S.Hadar*5.4 *S.infantis* the important serotype that cause outbreak in poultry but *S.typhimurium* and *S.enteritids* are the common is human infection in Japan, Australia, New Zealand and Europe .the current finding may indicated that *S.infantis* play role in food borne disease transmission by broiler meat, these idea was agreement with David et al 2001 who recorded that the Non typhoid salmonella were considered important food borne disease ,that cause public health problem worldwide, and *S.vichow* is one of these serotype also, in Europe and Switzerland, *Salmonella vichow* is among 5 of most serovars frequent isolated from poultry and these strain induced severe invasive infection .Also the isolated *S.virchow* from

broiler meat in the current study was in consistent with Iran et al.,2013 who recorded that among 169 *Salmonella* spp isolated from meat of Turkey, *S.vichow* form 12%, of these serotypes It was found that the most important third bacterial isolates in current result is *S.entritidis*, these result was agreement with result of Medeiros et al.,2011 who recorded that the most frequent species of *Salmonella* isolated from poultry meat, is *S.enteritidis* (48.8%) followed by *S.typhimurium* (7.2%) ,also High prevalence of *S.enteritidis* isolated from poultry meat was reported in varies countries, in USA, (Altekruse et al 2006) recorded that during 2000 to 2005,significant high prevalence of *S.enteritidis* in chicken carcasses, also these serotype was the predominant isolated from poultry meat in the Brazil (Duarte et al 2009). Also Altekruse et al 2006 found that *S.enteritidis* is the main isolates from Turkey meat samples, The present finding expressed that *S.hato* was isolated from broiler meat, these may be the broiler meat contaminated with broiler feces in slaughtering process.

Assèta et al.,2013 recorded that 22 out 350 poultry fecal samples express positive *S. hato* isolates also they found that *S.enterica*. The isolated of *S.dublin* from broiler meat may indicated that the broiler particular live broiler may infected with *S.dublin* and the chicken carcasses may contaminated by these organism during opening their digestive tract. in the abattoirs ,the main source of contaminated carcasses by *Salmonella* is the opening of GIT during slaughtering food animals (Ayachi et al 2010), in addition, pet animals asymptomatic carriage *Salmonella* in these feces and they spread these MO in the environment,(Glaser et al 1994). on the base above, we suspected that cause of isolated *S.dublin*, a specific pathogen for cattle, in broiler meat,that due to roam free the raw chicken that transient infection with these pathogen.

the current result showed that *S.infantis* highly (100%) resistant to PRL10, CTX10 and moderate resistant to CIP10,AK10 \and CN10, these result may indicated that these serotype are multidrug resistant that it resisted to common antibiotic using in the treatment of Gastroenteritis which including third generation, these result was similar to observation of Martin et al 2004 who reported increasing *Salmonella* serotypes resistant to most important medically antibiotic such third generation cephalosporins, quinolones and fluoroquinolones and found that the active antibiotic using in the treatment of *Salmonella* spp are fluoroquinolones, macrolides and third generation

cephalosporins. in the human,previously,it was reported that *Salmonella* strains isolated from human were sensitive to cephalosporine and less percentage of avainorigin strans can resistant these drugs in USA but later on,both human and avain origin *Salmonella* strain expressed high resistance to cetiofur with decrease susceptibility to ceftriaxone (Stopforth et al 2006).

The high prevalence of antibiotic *Salmonella* strains in food lead to induced outbreak of salmonellosis as in the Brazil (Food and Drug Administration 2006)the high prevalence of antibiotic resistant *S.infantis* in the present study may indicated these serotype of *Salmonella* may cause important economic and public health problem in Iraq, Multiantidrug bacterial resistant cause important public health and economic problem worldwide, the source of antibiotic resistant *Salmonella* coming from food origin animals ,wide spread antibiotic resistant bacteria occur due to mistreatment of human with antibiotics as well as usage antibiotic as growth promoters in livestock , therefore the most source of antimicrobial *Salmonella* serovars is farm animals (Duarte et al 2009).

the recorded multi antibiotic resistant of *S. Thompson* in the current study was agreement with Ezekiel et al. 2011 who found that *Salmonella* spp isolated from chicken meat was 100% resistant to ugmentin also these pathogen was 90-100% resistant to tetracycline (Sakaridis et al. 2011).

The present study is considered a first research in Iraq, that revealed the percentage of certain Zoonosis bacteria particularly *S.infantis* in local and imported frozen and raw broiler meat at local markets and antimicrobial resistant of *S.infantis*isolates.these result also supported idea that the main source of food borne disease is the animal products..

The isolation of *S.infantis* in the present study with multi drug resistance expressed by these pathogen may indicated these strain play important role in the broiler meat poisoning that lead to increase hospitalizations with economic costs in addition mortality particularly in immunocompromized patients, therefore, must be frequently evaluated the presence of *S.infantis* in poultry meat in the local markets in Iraq, in order to control the infection by these pathogen particularly the study about *S.infantis* were very little in Iraq. Ezekiel et al 2011found that increased circulation of multidrug resistant

Salmonella species associated with mortality and hospitalization as well as economic costs.

Other important Zoonosis bacteria isolated from frozen broiler meat in the present study were *E.coli* (99%), the present finding may indicated that the source of broiler meat were unfit for human consumption and these meat was considered a source of *E.coli* that may lead to food poisoning and gastroenteritis in human population.

The presence of *E.coli* in the meat poultry may indicated that the meat contaminated with intestinal containing due to these organisms are faster antidrug resistant as compared with other bacteria (Miranda et al. 2007), therefore *E .coli* commonly isolated from poultry meat due to contaminated the meat through slaughtering ,processing and handling (Canton et al. 2008; Adesiji et al. 2011),as well as a result of widely usage of antibiotics drugs (Miranda et al. 2007),however, due to *E.coli* is a normal flora of intestine of warmed blood animals and humans, the presence of these organism in the meat is a good indicator of fecal contamination (Mead, 2000) the present percentage of *E.coli* isolates was low as compared with those reported by Adesiji et al. 2011 and Ukut et al. 2010) and 60 % respectively the, isolation and identification of *E.coli* was dependent on the ways and type of media using in the isolation, therefore the prevalence of these bacteria was different from countries to other, Dahal, 2007 reported that 19% of these organs in meat poultry in the South Africa while Saikia and Joshi, 2010reported high prevalence of *E.coli* (98 %)isolated from meat poultry in India The current finding revealed among 100 broiler meat samples .8,14,7 were positive for *Pseudomonas* spp, *Citrobacter* spp and *Proteus* spp respectively. The present result may indicated that the broiler meat were contaminated by these organism which considered normal flora in the chicken . Contaminated meat may occur by contaminated knives which used in the cutting meat into smaller parts, from environment and during chopping meats to mince (Unluturk and Turantas, 1999).also contamination come from normal floras organism in poultry such as *E.coli*, *Pseudomonas* (Mead, 2000).

The current finding may supported idea that poultry meat is important source of food born disease ,these evidence was agreement with Conner et al., 2000 who demonstrated that 95% of food borne disease induced by meat of chicken in the developed countries such as Australia, Canada and UK It was concluded that the frozen broiler meat in local markets were considered

important source of *Salomella* spp particularly multi drug resistant *S.infantis* and other food borne pathogens.

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