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Determination the Percentages of Microbial Contaminants in Crops and Livers by Using the Traditional and Backtrac 4300 Technology

[1]Sudad J. Mohammed, [2]Doaa Adel Qasim, [3]Adil .T. Al – Mousawi

***ABSTRACT*--** Poultry meat, like other foods, is subjected to contamination from microorganisms during the process of manufacturing, storing, marketing and selling them if health measures are not taken, makes them a cause of diseases for the consumer, especially food poisoning, so this study came to examine poultry meat (livers and crops) available in Baghdadlocal markets. Fourteen samples of chicken meat were collected during 2019, including 7 liver samples and 7 crop samples. A serial dilution was made to estimate the level of microbial contamination including (*coliform* bacteria, *Staphylococcus aureus*, and *Salmonella spp*) by using traditional method and Back Trac technique.in all chicken samples*Staphylococcus aureus* were detected, where the highest contamination in sample (Al-kawther) was (9×10^4) CFU/g in the livers while in crops was (6×10^4) CFU/g. The highest contamination with *coliform* bacteria was in the (Hanana) sample, in the livers was (8×10^2) CFU/g while in crops was (4×10^2) CFU/g, the results also indicated the samples of (Al-Bayader) and (Al-kawther) was positive for *salmonella* bacteria. While after examining the same samples with BackTrac device, the highest bacterial count was found in a sample (Al-kawther) when examining the bacteria of *staphylococcus aureas*, where the highest percentage (3.4 E+5) was in the liver, but in the crops the total count was (1.8 E+5) and the detection time was started at 8.80hrs, while *coliform* bacteria, was also found in a sample (Al-kawther), where the total number in livers (4.6 E+3) while in crops was (2.4 E+2),the detection time was started at 11.33hrs, the samples (Hanana) (Al-Bayader) (Al-kawther) were positive for detection the *salmonella* bacteria and the detection time was at 5.96 hrs.

***Keyword*--**Microbial Contaminant, Liver and Crops products, Back TracTechnology.

I. INTRODUCTION

Zoonotic diseases transmitted to humans by food are among the most important health problems and there are more than 200 diseases transmitted to humans through contaminated food. These diseases differ in their health and economic importance, there are many diseases transmitted by Poultry meat that are referred to as zoonotic emergency diseases have appeared in recent years because it spreads through food suddenly and widely, including *Salmonellosis*, (Santos*et al*., 2003).

Chilled and frozen chicken livers are one of the types of chicken meat available in the local and international markets and have an increased demand as a result of a its lower price compared to the rest of the types of meat as well as it contains unsaturated fatty acids with high levels of long-chains as C18: 1 percentage of fatty acids and C18: 2 more From 53% of the total percentages of total fatty acids, in addition to the high percentage of protein,

[1] Center for Market Research and Consumer Protection/ University of Baghdad

[2] Center for Market Research and Consumer Protection/ University of Baghdad

[3] Center for Market Research and Consumer Protection/ University of Baghdad

as it contains high amounts of vitamins and minerals, which made it an essential nutrient in our daily life, (Abo-salem and Abou.Arab, 2010).

During the preceding years, many types of frozen chicken livers were brought to the local consumption in the country, from different local as well as international markets, and most of them were almost not subject to the health control, as chicken meat with all kinds of food is perishable if preserved or stored in Inappropriate conditions, due to the presence of various numbers and types of microorganism on the meet surface, the number of these microorganisms varies according to several factors, the most important of which is the extent of commitment to apply health conditions during transportation, storage, and handling, (Al-Hemair, 2011).

Frozen chicken carcasses may be exposed to melted during marketing operations or when displaying in stores (Christian and stephen, 2010) Thus, the number of spoilage bacteria increases in chicken carcasses as a result of the preparation of human slaughterhouses makes chicken inadequate for human consumption (James*et al.*, 2006)

The microbial quality of poultry meat is affected by the impact of pre-slaughtering operations, where increase of a large number of pathogenic bacteria was observed in the intestines and chicken meat that exposed to thefodder before being transferred from slaughterhouses, Thanigaisal and Anandhan (2015).

The owners of food processing factories have a social responsibility as they must make sure that the food that they manufacture and sell will not cause harm to the health of the consumer, by accomplishing several steps related to cleaning and buildings belonging to the factories and not just content with adding preservatives that are harmful to the health of the consumer and may also lead to withdrawal One of the food products from the market as a result of its pollution to cause harm to the producing company and society, in addition to poor public health (Mandell*et al.*, 2010).

As a result of the weakness of the control, standardization and quality control agencies due to the political and economic conditions that the country is exposed to and the increase in trade exchange, many companies began importing many types of frozen meat from different origins, so this study went to assess the quality and the microbial quantity of some types of imported and local chicken found in local markets. To determine its suitability for human consumption, the study also recommends periodic tests to protect the Iraqi consume.

The purpose of this study was to analyze the level of contamination of frozen chicken livers and Crops that present in the local market by estimating the total numbers of *Escherichia coli*, *salmonella* spp and *Staphylococcus aureus*, which are important indicators and microbial evidence for measuring the level of bacterial contamination in comparison with BackTrac Technology

II. MATERIALS AND METHODS

Collection and preparation of samples:

Samples of chicken meat were collected during two periods, the first period during April and May of 2019 and the second period during September and October 2019, fourteen samples were collected including 7 liver samples and 7 crop samples. The samples were collected randomly with a weight of 250 g for each sample from different markets of Baghdad province and kept in ice pox until transferred to the microbiology laboratory under aseptic condition (25 g) of each chicken sample was weighed and A serial dilution was made with 225 mL of sterile buffered pepton water to homogenized sample up to 10^{-6} and plated into tested medium with duplicate to estimate the level of microbial contamination including (*coliform bacteria*, *Staphylococcus aureus*, and *Salmonella spp*).

Detection and Enumeration of bacteria with Traditional Methods

Estimation of total coliform bacteria

Violet Red Bile Agar (VRB- Himedia) was prepared according to the instruction company to enumerate the total *coliform* bacteria by poured 1 ml of diluted sample to prepared medium and another layer of media was added to make an anaerobic atmosphere, colonies were counted after incubated in 37˚C within 24 hrs.(Holt *et al*., 2002).

Enumeration theStaphylococcusaureus bacteria

Mannitol Salt Agar (MSA- Himedia) was prepared according to the instruction company and used for confirmation and enumeration of *Staphylococcus aureus* which is coagulase-positive, 0.1 ml of diluted sample has been spread with sterile spreader and the grown colonies were counted after incubated with 37˚C within 24-48 hrs.(Holt *et al*., 2002).

Detection of salmonella

(Three to five) g of chicken Meat samples were taken and homogenized with 10 ml of sterile peptone water and incubated for 18 hours at a temperature of 37 ° C after that one ml of homogeneous sample was taken and placed in sterile glass tubes containing 9 ml of rich medium (Selenite broth), *salmonella* was isolated after transferring 0.1 ml of solution to an selective medium (Salmonella-Shigella Agar) and 24 hour incubation at 37 ° C.(Holt *et al*., 2002).

Detection and Enumeration of bacteria with BackTrac 4300 Methods

BacTrac instrument setting:-

BacTrac instrument setting in accordance with the Manufacturer Company (SY-LAB) instructions described below for each test in these Tables:

Samples were inoculated with 10 ml BHI broth for cell counting, and incubated in 37oC for 24hrs.Bimedia (Broth media) was used for diluted samples in Tenfold Serial dilution, 9.0 ml of broth media was distributed in six measuring cell,then 1.0 ml of the diluted sample was added to the first measuring cell and then a serial dilution was made;1.0 ml is taken from the first tube and moved from tube to tube until the dilution had been completed and 1.0 mL was discarded from the last diluted tube, The inoculated measuring cells were incubated at 37˚C for 24 hours using the BacTrac instrument (BacTrac 4300, 2013).

Estimation of total coliform bacteria with BacTrac Instrument

BiMedia 160C was prepared as per Supplier Company's (SY-LAB) instruction which is a selective medium for *coliform* bacteria.and the setting program for BacTrac Instrument was illustrated in table (1):

Table 1. Setting Program for BacTrac Instrument.

Temperature	37 ˚C
(Durations times)	At least 12hrs
(Delay times)	one hour

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The Scales	M-values(5 to 30) %
	E-values(5 to 60) %
The Thresholds	M-values(3) %
	E-valueswas not considered

Enumeration of Staphylococcus bacteria with BacTrac Instrument

For detection of *Staphylococcus aureus*, samples were pre-Enriched previously with preMedia 350A which is a selective pre-enrichment Medium for *Staphylococcus aureus* by incubate 1ml of the homogenized sample in 9ml (preMedia 350A) for 24hours at 37˚C, after incubation, 0.1 ml of pre-enriched sample was tacked and inoculated with measuring cell containing 9.9 ml of selective Medium (BiMedia 350A) and incubated for 24 hours at 37˚C and the setting program for BacTrac Instrument was illustrated in table (2):

Table 2.Setting Program for BacTrac Instrument.

The Temperature	**37 ˚C**
(Durations time)	24 hrs
(Delay time)	one hour
The Scales	M-values (5 to 30) %
	E-values (5 to 80) %
The Thresholds	M-values was not considered
	E-values (10) %

Detection of salmonella with BacTrac Instrument

BiMedia 205 A which a modified Selenite Cystine medium was used as pre-enrichment media for homogenized samples by incubated 1ml of sample with 9ml pre-enrichment media for 16-18 hours at 37˚C, after that 0.1 ml from pre-enrichment media was taken and inoculated with 9.9 ml from BiMedia 201 C which a selective media for *salmonella* and incubated for 24 hours at 40˚C and the setting program for BacTrac Instrument was illustrated in table (3):

Table 3. Setting Program for BacTrac Instrument.

The Temperature	40 ˚C
(Durations time)	24 hrs
(Delay time)	one hour

The Scales	M-values (2 to 10) %
	E-value (5 to 80) %
The Thresholds	M-values (3)%
	E-values (10) %

Statistical Analysis

Statistical analysis information was analyzed using computerized variance testing and multi-range test procedures by DuncanStatistical Methods, (Oxford, 2013).

III. RESULTS AND DISCUSSION

(Table 4) displays the results for samples indication card analysis that obtained from local markets, by specifying the trademark of the produced chicken, the type of packaging, its weight, the country of origin, the production, and expirydate.

Table 4. The collectedsamplesand its information

No	Sample name	Origin	Date of production	Date of expiry	Volume /gram	Notes
1	Mayda	Emirates	2018/12/30	2019/12/30	250	Frozen chicken
2	Hanana	Iran	2018/12/24	2019/12/24	250	Frozen chicken
3	Bakpi	Turkey	2018/11/10	2020/2/10	250	Frozen chicken
4	Al-Bayader	Jordan	2018/12/1	2020/3/6	250	Frozen chicken
5	Randa	Emirates	2018/12/20	2020/3/18	250	Frozen chicken
6	Sadia	Brazil	2018/12/7	2020/3/6	250	Frozen chicken
7	Al-kawther	Iraq	2018/12/3	2020/3/6	250	Frozen chicken

It has also been observed from the date of collecting samples that the collection has been divided into two periods of the year, which are March and April of the year 2019, and after September and the first of the year 2019, so that in these months, temperatures decrease in Iraq, where the growth of microorganisms is in a balanced manner, as the periods In other parts of the year,

Temperatures rise, as well as the conditions of power outages in Iraq, especially for retail stores. The shelf life will decrease significantly and lead to rapid damage to the product, which may give negative results to microscopic examinations of samples, On the other hand, looking at the expiration period indicates that all samples were given a validity period of one year, and in this case, most of the samples produced were in December of 2018, which is an approximate period for the first period of collection, but the expiration date was most in March for the year 2020, which is the period that is close to the second collection period before the expiration date, and therefore,

the remainder of the validity period will be eight months, which is the period that was specified on the media indication card under storage conditions under freezing.

Results of Detection and Enumeration of bacteria with Traditional Methods

The results shown in (Table 5) indicated the presence of microbial contamination in all samples that subjected to the examination, where the presence of *Staphylococcus aureus* bacteria was observed in all samples, while the *coliform* bacteria was observed in examined samples of (Hanana, Al-Bayader, and Al-kawther), while the samples of (Al-Bayader and Al-kawther) were contaminated with *Salmonella*.

Table 4. Enumeration of bacteria with Traditional Methods

No	Sample name	*Staph. aureus* CFU/g		LSD value	*Coliform* bacteria CFU/g		LSD value	*Salmonella* spp. CFU/g	
		Livers	Crops		Liver	Crops		Liver	Crops
1	Mayda	2×10^3	8×10^2	46.38 *	-	-	---	-	-
2	Hanana	1×10^4	4×10^4	41.07 NS	8×10^2	4×10^2	16.52 *	-	-
3	Bakpi	4×10^1	2×10^1	22.42 NS	-	-	---	-	-
4	Al-Bayader	3×10^3	4×10^3	17.06 NS	-	1×10^1	---	Positive	-
5	Randa	2×10^2	6×10^2	35.71 *	-	-	---	-	-
6	Sadia	6×10^2	8×10^1	33.08 *	-	-	---	-	-
7	Al-kawther	9×10^4	6×10^4	28.33*	5×10^2	9×10^1	37.62 *	Positive	-
	LSD value	52.49*	39.62*	---	32.74 *	27.91 *	---	---	---

It was found through the results obtained, there was microbial contamination in all samples, in this results *Staphylococcus aureus* were detected in all chicken samples, where the highest contamination of the samples was recorded in the sample (Al-kawther), as the total number of bacterial colonies was (9×10^4) CFU/g in the livers while in crops the total number was (6×10^4) CFU/g, while the lowest percentage of contamination with *staph* bacteria was in the sample (Bakpi), where the contamination rate (4×10^1) CFU/g was in the livers sample, but in the sample of crops it was (2×10^1) CFU/g, and this result corresponds to the results of (Thanigaisal and Anandhan, 2015) With some variations in numbers,However, the results were within the microbial limits permitted by the Iraqi Standard (Central Organization for Standardization and Specific Control), the Iraqi Standard for Frozen Chicken No. 1179 of (1989).

For the examination of *coliform* bacteria, it was found that there are some samples were contaminated with *coliform* bacteria. The percentage of contamination in crops was more than in livers samples, where the highest contamination rate was in the (Hanana) sample, where the percentage of bacterial contamination in the livers was (8×10^2) CFU/g while in crops was (4×10^2) CFU/g, While the lowest contamination rate was in a sample (Al-kawther) where the bacterial count of *coliform* bacteria in the livers reached (5×10^2) CFU/g while in crops samples was (9×10^1) CFU/g. The remaining samples were free from bacterial contamination with coliform bacteria; these results were close to what (Al-Hemair, 2011) referred to in his results, (Alsoufi *et al*., 2016) indicated that the highest total number of bacteria in imported frozen chicken thighs obtained from local markets was (16×10^3) CFU/g while the lowest number (1×10^3) CFU/g. The reason for the presence of this contamination may be due to the adoption of incorrect storage conditions during import or circulation until reaching The consumer, in addition to

the large number of its melting, as the melting process contributes to providing encouraging conditions for the growth of this group of microscopic neighborhoods and then deteriorates its quality and damage over time,(El Shrekand Ali, 2012) or this contamination came from the waste water of manufactures Which is loaded with many types of multidrug resistance bacteria such as *faecal coliform* and *Faecal streptococci* (Kuljinder and Kahlon, 2016)

However, the results also indicated that the samples (Al-Bayader) and (Al-kawther) was positive for*salmonella* bacteria, which are a dangerous bacterial species, which stated the Iraqi standard specifications for frozen chicken (Central Organization for Standardization and Specific Control), the Iraqi Standard for Frozen Chicken No. 1179 of (1989), that they do not exist in frozen chicken due to their risk to the health of the consumer, as all references related to this subject indicate that Food is devoid of these bacteria even if they are in small numbers, as the presence of only one of them leads to food rejection and is considered unhealthy food and should not be consumed, Hannon, R (2013)indicated that the reason for the presence of these bacteria is due to the lack of safety of workers or the safety of devices, equipment, packaging materials and packaging from The successor Health, as well as the possibility that the chicken itself is carrying bacteria, however it is slaughtered, marketed and consumed.

Results of Detection and Enumeration of bacteria with BackTrac 4300 Methods

BacTrac 4300 was used in this study for detection of growth phase for detected bacteria which is an Austrian device and the first instrument in the (market research and consumer protection center/ Baghdad University), whichconsiders a rapid microbiology system automatically, detected or enumerated a wide range of micro-organisms with highly selective broth media for each micro-organism.

In this study the determination of growth phase with BacTrac 4300 illustrated that the detection time of *staphylococcus aureas* was at 8.80hrs while the stationary phase at 13hrs in which the phase of production of toxins that caused the food poisoning.

Figure (1) showed the control results which include (broth media without samples), Whereas growth curve result shown in Figure (2), the growth of *staphylococcus aureas* in broth media selective Medium (BiMedia 350A) for 24 hrs, In which the time of detection of bacterial cells in broth media began at 8.80 hours, which reflected the stage of cell division and its presence in the broth media with the end stage of (lag phase), before the growth started to reach the stage of (stationary phase) at 13 hours.

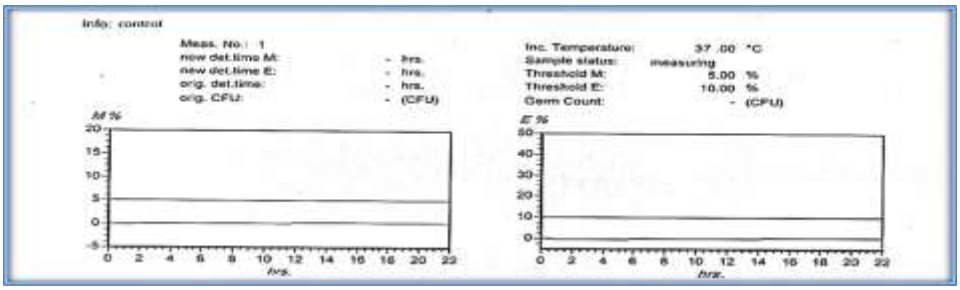

Fig 1. The control result (without samples) in Broth media (Bimedia)

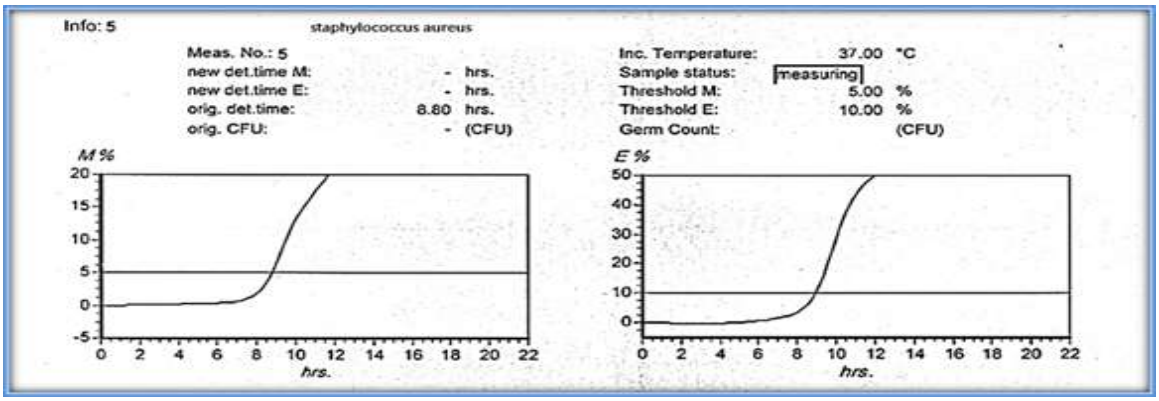

Fig 2. Curve of Growth of*staphylococcus aureas* using broth media selective Medium (BiMedia 350A) with (BacTrac 4300)

While the detection time of *coliform* bacteria was at 11.33hrs while the stationary phase at 15.30 hrs. The result of growth curve shown in Figure (3) the growth of *coliform* bacteriain BiMedia 160C which is a selective medium for *coliforms* bacteria for 24 hrs, In which the time of detection of bacterial cells in broth media started at 11.33hrs, reflecting the stage of cell division and its presence in the end stage of broth media (lag phase),Until the growth begins at 15.30 hours, (stationary phase).

The period of growth and appearance of *coliform* bacteria took more time than the appearance of *Staphylococcus aureus*, which indicates that the percentage of *coliform* bacteria present is less than the proportion of *Staphylococcus aureus.*

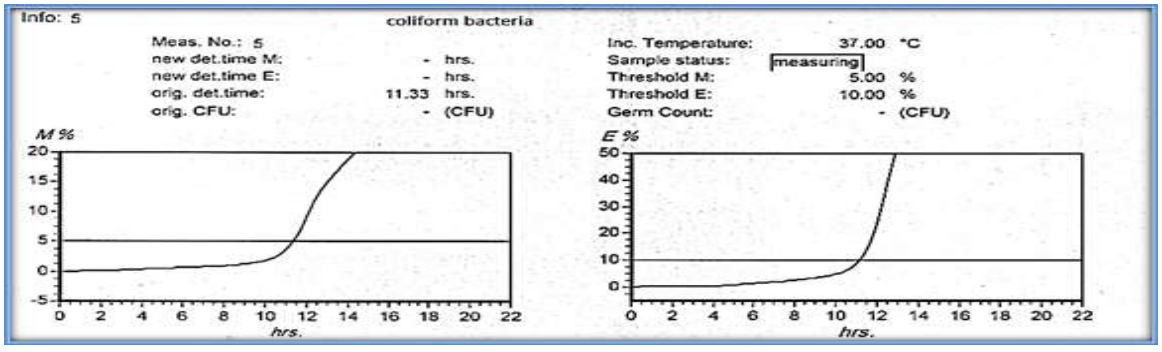

Fig 3.The curve of growth of *coliform* bacteria using broth media selective Medium (BiMedia 160C) with (BacTrac 4300)

However, the results also indicated that the samples were positive for *salmonella* bacteria, the detection time of *salmonella* bacteria was at 5.96 hrs while the stationary phase at 8.45 hrs. The growth curve result was shown in Figure (4) the growth of *salmonella* bacteria in BiMedia 201 C which a selective media for *salmonella* and incubated for 24 hours at 40˚C.

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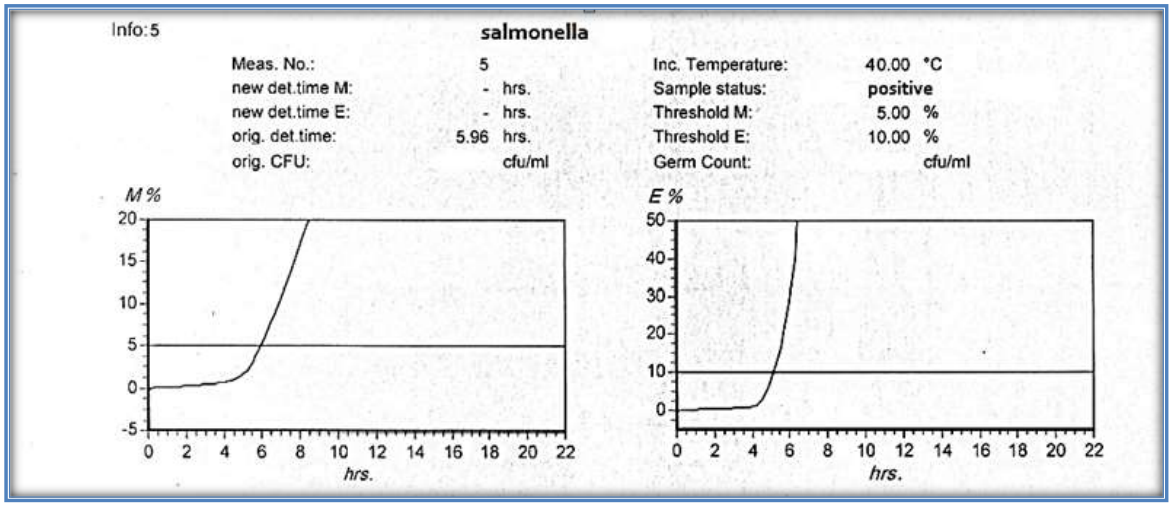

Fig 4. The curve of growth of *salmonella* bacteria using broth media selective Medium (BiMedia 201C) with (BacTrac 4300)

Following serial dilution of the bacteria that shown in Table (6) the Detected Bacteria Colony Forming Unit (CFU) was determined, BiMedia which consider a selective media for detection and enumeration was used for each type of bacteria, the highest bacterial count was found in a sample (Al-kawther) when examining the bacteria of *staphylococcus aureas*, where the highest percentage (3.4 E+5) was in the liver, but in the crops the total count was (1.8 E+5)

The lowest bacterial count was in a sample (Bakpi) in which the percentage of bacteria was (16.4 E+1) in the livers samples, but in the crops sample, the bacterial count was (2.4 E+2).

As for the detection of the presence of *coliform* bacteria, it was the highest amount of contamination in a sample (Al-kawther), where the total number in livers reached (4.6 E+3) while in crops was (2.4 E+2), As for the rest of the samples (Mayda) (Bakpi) (Randa) (Sadia), it was found that they are free from the presence of *coliform* bacteria in both livers and Crops.

With regard to the detection of *salmonella* bacteria, the BacTrac device was more accurate than detection by traditional methods. It found contamination of more than one of the examined samples that were examined whether they were livers or crops, the samples (Hanana) (Al-Bayader) (Al-kawther) were positive for detection the *salmonella* bacteria this results was accordance to (Sudad J. Mohammed, 2016)

Table 6. Determination of CFU of detected bacteria with BacTrac.

NO	Sample name	*Staph. aureus* CFU/g		*Coliform* bacteria CFU/g		*Salmonella spp.* CFU/g	
		Liver	Crops	Liver	Crops	Liver	Crops
1	Control	-	-	-	-	-	-
2	Mayda	8.4 E+3	2.3 E+3	-	-	-	-
3	Hanana	6.3 E+4	14.6 E+4	12.4 E+2	6.2 E+2	Positive	-
4	Bakpi	16.4 E+1	2.4 E+2	-		-	-

5	Al-Bayader	6.4 E+3	3.6 E+4	3.2 E+2	1.1 E+2	Positive	Positive
6	Randa	13.4 E+2	1.4 E+3	-	-	-	-
7	Sadia	11.2 E+2	5.8 E+2	-	-	-	-
8	Al-kawther	3.4 E+5	1.8 E+5	4.6 E+3	2.4 E+2	Positive	Positive

E+1= 10^1 of dilution

IV. CONCLUSION

The current study reveals the fact that raw chicken material was heavily contaminated with bacterial pathogens. Food contaminated with bacteria causes diseases transmitted to humans and causes digestive disorders that may threaten the life of the consumer. Despite the researcher's inability to dispense with traditional methods of detecting bacterial contamination in foods, modern methods and devices remain the best and most accurate in detecting the presence of pathogens that help the researcher to accomplish the work with the lowest costs and the fastest time and best results, in this research it was found that Using a BacTrac device is better in giving and determining results than traditional methods, especially in examining foodstuffs because it gives results to the examiner and the consumer as soon as possible and more accurate

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