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Isolation and identification Salmonella *Spp.* from the diarrhea cases in chicken by classical methods in AL-Diwanyia city

A Research
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بسم الله الرحمن الرحيم

(فَتَعَالَى اللَّهُ الْمَلِكُ الْحَقُّ ۗ وَلَا تَعْجَلْ بِالْقُرْآنِ مِن قَبْلِ أَن يُقْضَىٰ إِلَيْكَ وَحْيُهُ ۖ وَقُل رَّبِّ زِدْنِي عِلْمًا)

صدق الله العلي العظيم

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Certificate of Supervisor

I certify that the research entitled **(Isolation and identification *Salmonella Spp.* from the diarrhea cases in chicken by classical methods in AL-diwanyia city)** was prepared under my Supervison at the college of Veterinary Medicine/ University of AL-Qadissiya.

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13 / 4 / **2016**

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Thank god lord of the worlds may Allah bless Muhammad and aspects of my thanks and appreciation to my professors valued all of what have made me of valuable guidance opinions sound as Iam pleased to draw my thanks and gratitude to assist lecture Azhar Abdul sada.

Dedication

To .. my parents

And to all members of my family

To .. my friends

I dedicate this work

Ahmed

Summary

Abstract

Salmonella is one of the most important pathogenic bacteria that implicated in food –borne bacterial out breaks and diseases worldwide and constitute the potential public health hazard in many parts of the world . There are several transmission vehicles for Salmonellosis ,but the majority of human infections were derived from the consumption of contaminated foods especially those of animal origins such as red white meats . The main objective of the present study was, To isolated and identification of Salmonella *spp.* from diarrhea cases in chicken in Al- Diwaniya city by conventional culture methods.

A total of (30) diarrheic cases were obtained chicken were subjected to bacterial isolation on selective media (MacConky agar , Salmonella-Shigella agar , CHROM agar salmonella) 6 (20%) samples appeared the ability to isolation of *salmonella spp.* After that *salmonella spp.* isolates were subjected to biochemical reactions .the *salmonella* isolates were positive for, (citrate utilization , catalase and motility) but negative for (Indol and urease) in chickens infected with diarrhea .

List of Abbreviations

Salmonella	S.
Salmonella species	Salmonella spp.
Salmonella-Shigella agar	S.S. agar
Xylose lysine desoxycholate agar	XLD agar

List of Contents

List of Figures

List of Tables

Chapter One

Introduction

Introduction

salmonellosis : ONE of the most common infectious diseases in the world salmonellosis in both in humans and animals . The genus Salmonella are gram-negative and facultative anaerobic ,rod-shaped bacteria **(1)** chickens have been implicated as a major source of *Salmonella* contaminated food products as chicken eggs or meat that cause human salmonellosis **(2).**The isolation and identification of *salmonella* from clinical samples by traditional cultural techniques requires laborious procedures which can last up to 7 days In recent years ,diagnosis laboratories have been concerned with reducing the time required for the diagnosis of *Salmonella* infections and a more rapid and sensitive method for detection and identification of salmonella from chicken meat and food poisoning patient **(3)**.

Salmonellosis represents an important food borne disease It was estimated that approximately (70 % to 80 %) of food borne bacterial outbreaks **(4)** In humans. *Salmonella* represent important pathogen in food poisoning, although it has a much lower prevalence in farm animals, compared with pathogenic *E. coli* and *Campylobacter* **(5)**. that continues a major and unacceptable threat to human public cases , *S. typhimurium* is linked to a range of food-producing animals such as poultry, swine, cattle and sheep. *S. enteritidis* was a rare serovar until the mid-late 1980s when it emerged as a frequent cause of Salmonellosis in European countries and across the globe **(6)**By the 1990s, *S. enteritidis* replaced *S. typhimurium* . as the most common serotype of Salmonellosis isolated from humans in many countries **(7) .**

Salmonella infections are usually acquired via the food chain as a result of the ability of *Salmonella* servers to colonize and persist within the gastrointestinal

tract of their host. Proper hygiene and enforcement of public health is the key to prevent *Salmonella* food poisoning . the aims of the present study were the -: following

To Isolated and identification of Salmonella *species* from diarrhea cases in chicken in Al- Diwaniya city by conventional culture methods .

Chapter two

Literature Review

2- Literature Review:-

2-1 Taxonomy

The *Salmonella* genera belong to the *Enterobacteriaceae* family. The primary basis for the classification and identification of these bacteria has been a serological scheme, invented by White and refined by Kauffmann and others. Based on the serological identification of O (somatic) and H (flagella) antigens, Kauffmann concluded one serotype-one species concept, which was the reason why many serotypes were originally named by italicized Latin binomials (e.g. *Salmonella typhimurium*) **(8)**. In 1973, Crosa *et al.* demonstrated with a DNA-DNA hybridization method that all *Salmonella* servers are in a close relationship. After having this genetic evidence, it was approved that all salmonellas belonged to one single species, *enteric a serotype S. cholera suis*. The only exception was *Salmonella bongori*, which was proved to be a distinct species based on DNA-DNA hybridization **(9)**. Nowadays, two species of the genus *Salmonella* are accepted. (formerly subspecies V) and *Salmonella enterica*, which is composed of six subspecies: designated subspecies subsp. *enterica* (I), subsp. *salamae*(II), subsp *arizonae*(IIIa), subsp. *diarizonae*(IIIb), subsp. *houtenae*(IV) and subsp. *indica*(VI) and *Salmonella bongori* **(10)**.

(Table-2-1) *Salmonella* species, subspecies, serotypes, and their usual habitats, by Kauffmann-White scheme a Kauffmann-White scheme has been described elsewhere**(10)**.

***Salmonella* species and subspecies**	**No. of serotypes with sup Species**	
***S. enterica* subsp.*enterica*(I)**	**1,454**	**Warm-blooded animals**
***S. enterica* subsp.*salamae* (II)**	**489**	**Cold-blooded animals and the environment**
***S. enterica* subsp.*arizonae* (IIIa)**	**94**	**Cold-blooded animals and the environment**
***S. entericasubsp.diarizonae* (IIIb)**	**324**	**Cold-blooded animals and the environment**
***S. enterica* subsp.*houtenae*(IV)**	**70**	**Cold-blooded animals and the environment**
***S. enterica* subsp. *indica*(VI)**	**12**	**Cold-blooded animals and the environment**
S. bongori(V)	**20**	**Cold-blooded animals and the environment**
Total	**2,463**	

2. 2 . Morphology

Salmonella is Gram-negative, non-spore forming, facultative anaerobic bacilli, (2 – 3) µm in length and (0.4 -0.6) µm in size. *Salmonella* produce acid on glucose fermentation, reduce nitrates and do not produce cytochrome oxidase. Most of the salmonella are motile and do not ferment lactose. Different serotypes of *Salmonella* can be distinguished by the differential metabolism of sugars; *S. typhi* is the only serotype that does non- produce on sugar fermentation tests **(11).** To detect *Salmonella* from the stool sample, the clinical laboratories use low-selective media like MacConkey and deoxycholate agar and intermediate-selective media such as Salmonella-Shigella or Hektoen agar. Nowadays selective chromogenic media like Chromo agar is used for the primary isolation and preliminary identification of *Salmonella* from clinical specimens **(12).**

2.3.Epidemiology

However, *Salmonella typhi* and *Salmonella paratyphi* A do not have animal reservoir, therefore infection can be happened by eating the improperly handled food by infected individuals **(13).** Besides, transmission of *Salmonella* to the food processing plants and equipment's for food preparation are also of great importance. Once carried by vectors or transferred to food, consumption by human can result in the risk of Salmonellosis. The *Salmonella* cells can attach to food contact surfaces such as plastic cutting board which may develop into biofilm once attached and hence cause cross-contamination Consequently, *Salmonella* can enter the food chain at any point from livestock feed, through food manufacturing, processing and

retailing as well as catering and food preparation in the home **(14).** Disease surveillance reports frequently identify poultry (chickens, turkeys, geese and ducks) as the main vehicles in the Salmonellosis outbreak. **(15)** estimates 16 to 17 million cases occurred annually, resulting in about 600,000 deaths. The mortality rates differ from region to region, but can be as high as 5 to 7% despite the use of appropriate antibiotic treatment. On the other hand, non-typhoid cases account for 1.3 billion cases with 3 million deaths. In the United States, approximately 2 to 4 million cases of *Salmonella* gastroenteritis occur with about 500 deaths per year **(16).**

2.4. Salmonella genus

The genus *Salmonella* is composed of motile bacteria that are commonly found in the intestine of humans and animals, including birds and reptiles. Since *Salmonella* infects both humans and animals, bacteria are generally transmitted to humans through the consumption of contaminated food of animal origin, mainly meat, poultry, eggs and milk **(17).** *Salmonella* is a widespread illness-causing agent and the intestinal infections like Salmonellosis are common in people of all age, Salmonellosis is usually caused by *Salmonella* server *enteritidis* and symptoms typically include fever, diarrhea and abdominal cramps**(6)** . The other prevalent *Salmonella* infection is typhoid fever, caused by *Salmonella typhi*, which is highly endemic in Indian sub-continent and in other developing countries. *S. typhi* can invade into the bloodstream and cause life-threatening infections, like bacteremia. These severe generalized infections need to be treated with antimicrobials **(18 ,11)**Despite the great progress in medicine and food production, *Salmonella*

still remains Avery remarkable problem for public health, especially in the developing countries. Since the beginning of the 1990s, the multi-drug resistance (MDR) of *S. enterica* has emerged and during the last decade, the increase in the reduced fluoroquinolone susceptibility among *S. enterica* has become an even more important problem **(12)** .

2.5. Diagnosis of Salmonella :-

2.5.1. Cultural media :-

Isolation of the organism by culture media remains the most reliable method for detection, allowing precise identification of the bacteria and antimicrobial susceptibility testing, **(19)** both of which are critical for disease control. A variety of selective media which rely on visualization of simple biochemical features such as the non-fermentation of lactose and the production of hydrogen sulfide to identify *Salmonella* spp. The specificities of such media are poor, and time-consuming complementary testing is required to exclude as significant colonies of organisms with similar biochemical features **(20).** One of the latest techniques that used in recent decade to rapid detection of pathogenic agent in water and food is chromogenic media. chromogenic culture media first time introduced in 1979 by Ram Bach but it officially offered and produced since 1991, **(21)**. These media are very specific and their component act as substrate for specific enzyme and depending on enzyme exhibit special color . **(22)**.

2.5.2. Biochemical test :-

Typical *Salmonella* colonies based on morphology and or indicative biochemical reactions on selective agars are then cultured onto non-selective media prior to confirmatory testing. **(23)**. There are well-established confirmations and identification procedures for *Salmonella.* Preliminary identification is traditionally performed using classical biochemical and serological test **(24)**.Key biochemical tests include the fermentation of glucose, negative urease reaction, lysine decarboxylase, negative indole test, H2S production .

Chapter three

Materials & Methods

3- Materials and Methods:

3-1 Materials:

3-1-1 Instruments and Equipment's :

Table (3 - 1): Instruments and equipment's with their remarks

Instrument / equipment	Manufacturer / state
Autoclave	Mammert / Germany
oven	Mammert / Germany
Hot plate with magnetic stirrer	Heidolph (Germany)
Incubator	Mammert / Germany
Sensitive balance	Sartorius / Germany
Sterilized cotton swabs	Sterile EO. / China
Water distillatory	LapTech(Korea)
Petri dish	Al- Hani company / Lebanon
Test tubes	Al- Hani company / Lebanon
Light Microscope	Olympus / Japan

3.1.2 Culture Media :

Culture media used in this work are listed in Table (3-2).They were prepared according to the manufacturer' instructions on their containers and sterilized according to the suitable method.

Table (3-2): Culture media used with their remarks

Medium	Manufacturer (State)
Urea agar base	Hi media /India
MacConkey agar	Hi media /India
Xylose lysine desoxycholate agar(XLD)	Hi media /India
Salmonella-Shigella agar	Hi media /India
Nutrient agar	Hi media /India
Nutrient broth	Hi media/ India
Simmons's citrate agar	Hi media/ India
Chromo agar salmonella	Hi media /India

3-1-3 Solutions and Reagents:

A- Solution : Gram Stain (**25**) .

B- Reagents :

1- Catalase Reagent:

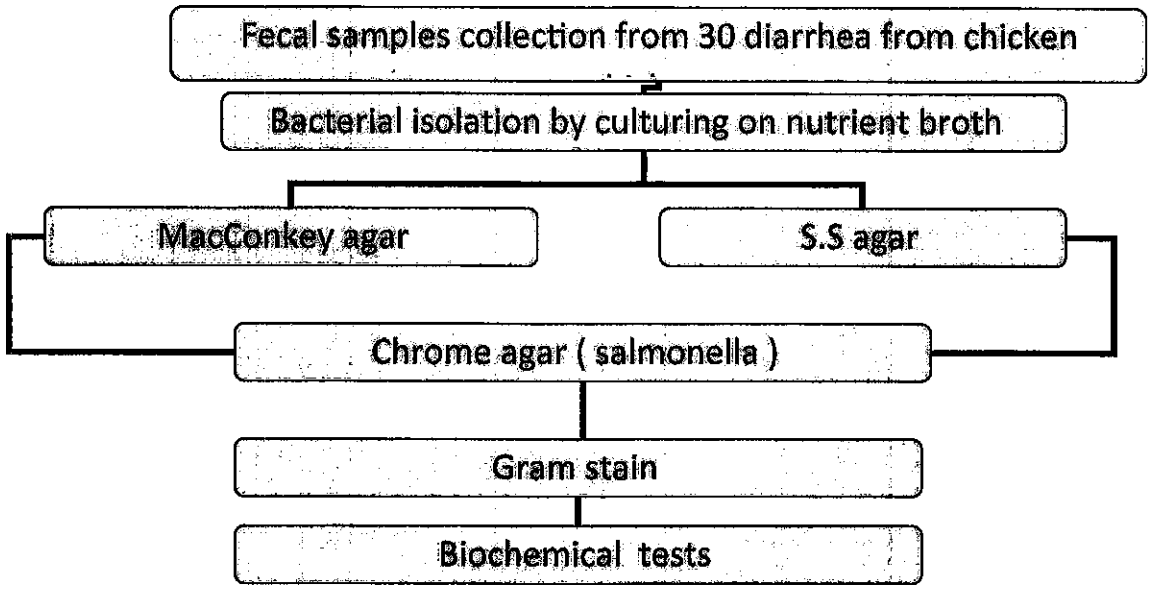
Hydrogen peroxide (3% H2O2) was used for detection the ability of bacteria to produce catalase enzyme **(23).**

2- Kava's Reagent:

This reagent was prepared by dissolving 10 g P-dimethyl-amino Benz aldehyde in 150ml is oamy alcohol and sol wily add 50 ml of concentrated hydro chloric acid (HCl),prepared in small quantities and stored in the refrigerator, shacked gently before its use. This reagent is used for detection of indole production.

3-2 Methods

3.2.1. Study design



3-2-1 Collection of Samples :

Took smears from diarrhea samples , A total of 30 smear from infected chicken with diarrhea by using Sterilized cotton swap assisted of the veterinary center and clinic or accruing in the city of AL- Diwaniyah , then transported to the laboratory in portable container to purpose bacterial culture .

3-2-2 Isolation and Identification of *Salmonella spp.*:

All fecal samples were cultured primarily on nutrient broth then incubated at 37C° for 24 hrs. . then culture was streaked on surface of , MacConkey agar and the positive growth subcultured on Salmonella-Shigella agar (S.S) then incubated at 37C° for 24 hrs. Then for more identification to isolate the colony subcultured on Chrome agar salmonella, then directly subjected to staining by Gram stain **(25).**

3.2.3. Biochemical Tests:

1- Indole Test:

Peptone water medium was inoculated with new culture colonies and at incubated at 37C°. for 24 hrs. , then few drops from Kovacs reagent were added . A red color in the alcohol layer indicated a positive reaction **(26).**

2- Simmons Citrate Utilization Test:

Medium was inoculated by streaking from saline suspension of the organism to be tested and Incubate for 24 - 48 hrs. at 37C Positive result was indicated by blue color and streak of growth while negative result was indicated by its green color with no growth **(26).**

3- Motility Test:

The motility test medium was inoculated with a straight inoculation, making a single state down the center of the tube to about half the depth of the medium . It was incubated under the conditions favoring motility incubation at 37C°. An examination was done after hours 1, 2 and 6 days **(26)**.

4- Urease Test:

The test was done by inoculation of urea agar medium with new culture colonies and incubated aerobically at 37C° for 24 hrs. A positive result was recorded by changing the color of the media from yellow to pink due to the ability of an organism to split urea , forming two molecules of ammonia by the action of the urease enzyme **(27)** .

5- Catalase Test:

A small amount of the bacterial growth was obtained and suspended in a drop of hydrogen peroxide 3% on a glass slide, and observed for evolution of bubbles as a positive result **(27).**

Chapter Four

Results & Discussion

4. Results and Discussion

4.1. Bacterial Isolation

All the salmonella *spp.* Isolates were able to produce bright colonies on MacConkey agar(fig. 4-1) , characteristic colonies produce black colonies on the XLD agar (fig. 4-2) , chromo agar salmonella the colonies characteristic Variable in size convex and mauve in color (Fig. 4- 3) . these results agree with **(28, 19).** Appeared results of the diagnosis 6 (20%) positive for salmonella spp. which get from (30) sample took from chicken infected with diarrhea Table (4-1). these results agree with **(29).**

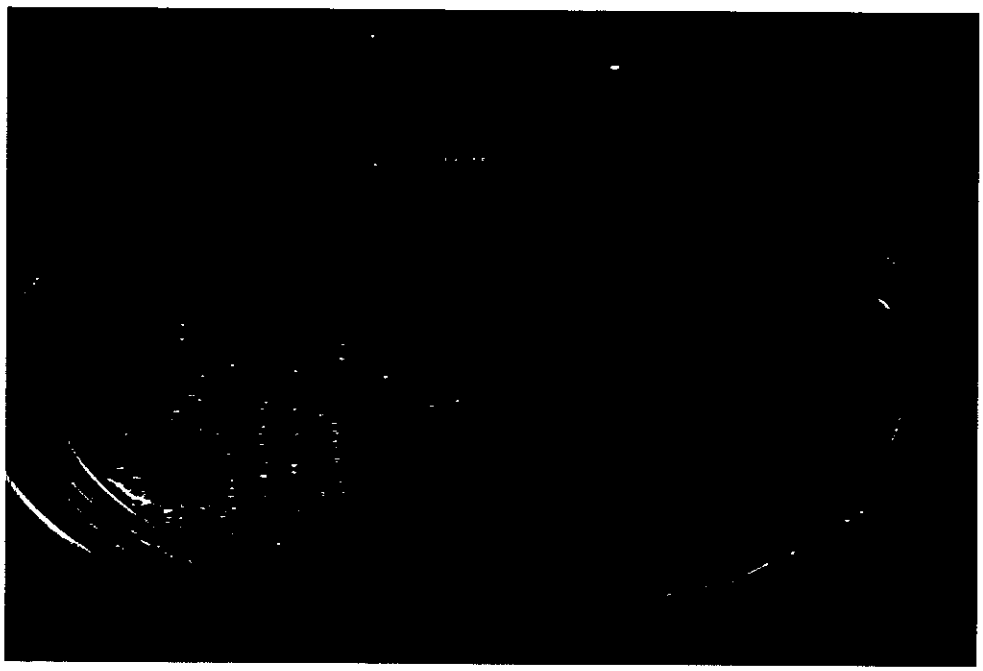


Figure (4-1) : characterization of *salmonella spp.* Colonies on MacConkey agar (bright Colonies).

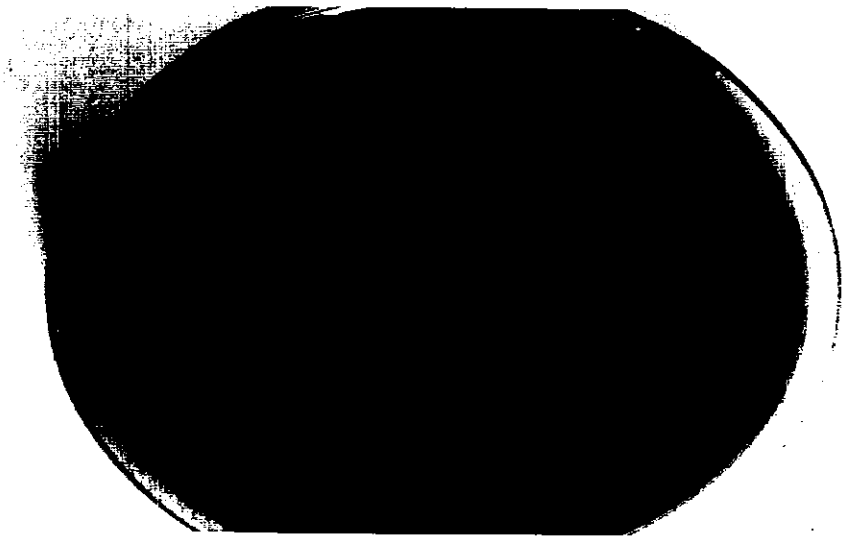


Figure (4-2) : characterization of *salmonella* spp. Colonies on XLD agar (black Colonies) .

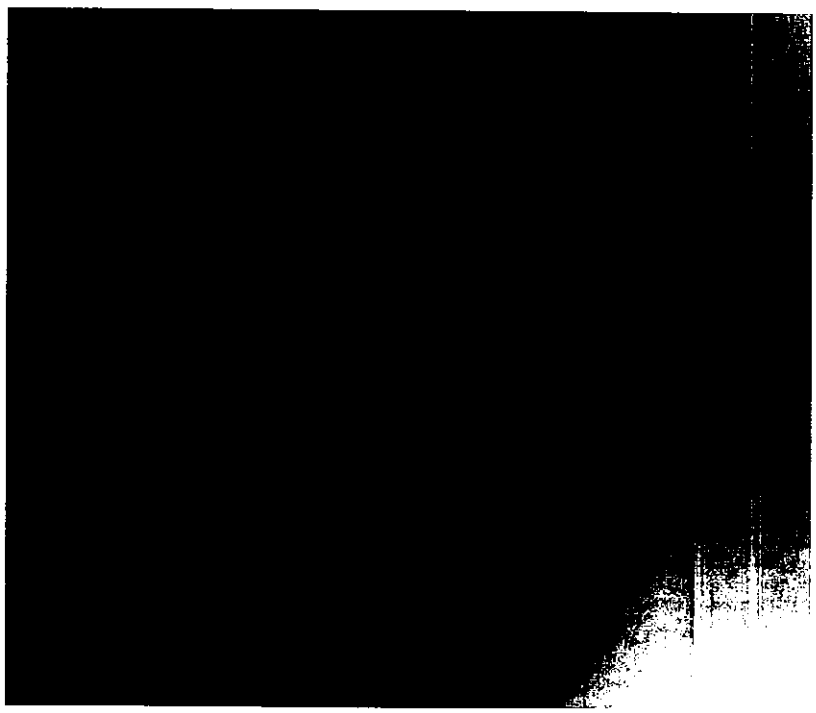


Figure (4-3) : characterization of *salmonella* spp. Colonies on Chromogenic salmonella agar (The arrow shows variable size mauve in color.)

Table (4-1) ; The prevalence of *salmonella* spp. Isolated from chicken

Samples	Number of Positive samples for salmonella spp.	%
30	6	20%

4-2 Results of Biochemical Tests

The identification of salmonella *spp.* Was confirmed based on their biochemical reaction that are Shawn in tables(4-2) . The biochemical tests for all isolates showed positive results to, citrate utilization as source of carbon, catalase methyl red and motility tests while gave negative reactions for indole production, urease. these results agree with **(30).**

Table (4- 2): Biochemical test results of *Salmonella* spp. Isolates

No.	Tests	Result
1	Citrate utilization	+
2	Indole production	-
3	Catalase	+
4	Urease	-
5	Motility	+

(+) Positive (-)Negative

Chapter Five

Conclusions & Recommendations

Conclusions:

1- high prevalence level of contamination with *salmonella spp.*in most chicken ,which are considered as the main source of food salmonellosis to humans being , when the most contaminated origins in chicken .

2- The contamination in the present study revealed was that loose the careful which supplied to chicken fields.

3- The Chromo agar *Salmonella* has the highest significantly sensitivity for identification of *Salmonella* spp. in sample comparison this media was effective for the isolation and presumptive detection of *Salmonella* in diarrhea samples .

Recommendations:

1- should be avoided dealing with chicken products imported from bad origin to protect the consumer . and Increased attention to health oversight of food products imported into the country, especially chicken products from different origin to protect public health.

2- The prevention of food borne Salmonellosis depends primarily on the careful handling of raw products and finished foods. each stage in the production, storage, processing, distribution and preparation of food may serve as a hazard or as an opportunity for prevention.