detection the pathogenicity of *Salmonella* spp isolated from drinking water in some region of Baghdad city in animal module.

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Abstract:

One thousand five hundred sixty seven water samples from different parts of Baghdad city were collected from the beginning of April 2010 till the end of December 2011, all samples were bacteriologically examined by traditional methods for detection of Total coliform and other pathogenic bacteria. Five isolate of *Salmonella* spp. were isolated and tested for its pathogenicity and ability to toxin production in the mouse module , all environmental isolates induced fluid accumulation (FA ratio≥100) after 5 hours and cause histopathological effects after 24h of inoculation. Histopathological changes showed inflammation of the mucosa and submucosa in the small intestine with mild chronic inflammatory cells and shortanage of villi ,mild degenerative of renal tissue and slightly necrosis, massive necrosis of hepatic cells with infiltrate of mild inflammatory cells in the liver section.

Introduction

Water borne diseases are caused by pathogenic microorganisms viruses, bacteria, intestinal parasites and other harmful microorganisms, which are directly transmitted when contaminated fresh water is consumed. Most of enteritis cases caused by drinking contaminated water with pathogenic bacteria such as *Salmonella* spp., determination of its virulence factors very important especially its ability to produce toxins. Nearly 2.2 million people die every year due to hygiene-related diseases, like gastroenteritis, typhoid fever and dysentery (Esha *et al.*,2009). Water sources are often contaminated with sewage and become the main causes of diseases such as typhoid and cholera , fecal contamination due to human and animal feces lead to the spread of *Salmonella spp.* in the surrounding environment and remain viable for months in soil, water and feces, which may be transferred to the community through drinking

water (Lehloesa and Muyima, 2000). Several methods have been used for isolation and detecting pathogenic bacteria and *Salmonella spp.* from water samples, such as traditional and molecular methods. The Maine objective of this study was the isolation of Salmonella from tap water by traditional methods and detection their pathogenicity in the animal organs

Material and Methods:-

1- **Water samples collection:-** One thousand five hundred sixty seven drinking water samples were collected randomly from houses in different parts in Baghdad area, from the beginning of April 2010 till the end of December 2011.
2- **Animals:-** Eighteen Sealed-adult-mouse model Swiss albino mice weighing about 15–20 g were used in this study to detected the pathogenicity of *Salmonella* spp., which accommodate from pharmaceutical care animals laboratory

Bacteriological Examination:- were carried out according to Eaton *et al.*, (2005).

Traditional methods:-

1- Isolation of of Total coli form(TC) , fecal coli form (FC), and *Escherichia coli (E.coli)* through using the membrane filter technique (MF), tube fermentation test (TFT) and presence /absence/ (P/A) method according to Delabre *et al.*, (1998) .
2- Isolation and Identification of *Salmonella* spp.:-
   1- The one liter of Tap water was filtered through a membrane filter with a pore size of 0.45 μm by vacuum pumping system.
   2- Place the membrane in flask with pre enrichment media **(**100ml**)** sterile peptone water, then incubated at 37 C° for 24h .
3- Loop full of positive growth peptone water was transferred for 100ml tetrathionate (x2) broth incubated at 37 C° for 24 h , streaked on selective media XLD or SS Agar then incubated for 24h at 37C° .
4- Suspected colonies were selected and further biochemical tests with API 20E and Mini Api were carried out.
5- The isolates which confirmed as *Salmonella* by biochemical tests, sent to reference for laboratory Central public health laboratory (CPHL) for serological conformation.

Detection of virulence factors in *Salmonella*:- which was performed by

1- **Animal model:-** The enteropathogenicity of *Salmonella* spp. was examined as described by Prasanta *et al.*,(2008) using the sealed-adult-mouse model Swiss albino mice weighing about 15–20 g. The animals, were kept in sterilized cages with autoclaved bedding, were

acclimated to laboratory conditions (12 h dark: 12 h light cycles; 24±1 °C). The procedure was carried out as following:-

1- The isolated strains were grown in brain heart infusion broth at 37°C with shaking for 24h, harvested by centrifugation.
2- Eighteen mice were divided into three equal groups ,the first group regarded as control, the second and third were consider as treated groups which were killed, depending on the time 5h ,24 h for detection the effects of the toxins on organs.
3- After 15 min, the bacterial inoculate (1×1^{10} CFU/ml) in 200 µl of Phosphate buffer saline were given to the test animal.
4- At 5 h from post- inoculation , the animals of second were sacrificed, and the fluid accumulation (FA) ratios were determined, FA ratios of ≥100 were considered positive.
5- For the colonization assay, infections were allowed to proceed for 24 h. The mice of third group were sacrificed, and the intestines, stomach, kidney, and liver were removed and kept for histopathological examination.

Histopathological examination:- Histopathology was performed as described by Chang and Miller (2006). After 24 h post-inoculation, mice were euthanized and sections of small intestine, stomach, kidney and liver were immediately fixed in 10% neutral buffer formalin. Following fixation, tissue samples were embedded in paraffin, sectioned at 5 µm and stained with haematoxylin-eosin for light microscopic examination.

Result and Dissection:-

1- Analysis of Drinking Water Samples:- Large variety of bacterial pathogens including *Enterobacter* sp., *Proteus mirabils*, *E.coli*, *Aeromonas hydrophila*, ,*Psedomonas sp.* and *V. cholera,* in addition to the Total coliform and Fecal coli form were isolated from drinking water samples as shown in Table (1).

Table (1) : Bacterial population isolated from drinking water samples in some parts of Baghdad area .

Months 2010	No. of samples	%of polluted samples	No.of samples polluted with Total coliform	*E.coli*	*Salmonella spp.*	*V.cholera*	*Pseudomonas spp.*	*Protus mirabils*	*Aeromonas hydrophila*	*Enterobacter cloacae*
4	34	88.2	30	29	-	-	2	-	3	3
5	150	56.6	85	62	1	1	4	6	-	10
6	120	50	60	51	1	1	5	4	-	6
7	155	40	62	42	-	-	1	5	2	4
8	105	47.6	50	35	-	-	-	-	-	2
9	75	60	45	22	-	-	-	1	-	4
10	75	56	42	26	-	-	1	2	3	18
11	90	43.3	39	16	-	-	-	-	-	2
12	45	44.4	20	7	-	-	-	-	-	2
2011										
1	72	44.4	32	15	-	-	1	-	-	-
2	53	49.1	26	7	-	-	3	-	-	4
3	54	64.8	35	27	-	-	1	2	-	25
4	73	64.3	47	30	-	1	2	3	1	4
5	100	72	72	48	-	-	3	4	-	4
6	65	63.1	41	29	1	-	1	2	-	5
7	56	48.2	27	13	2	-	-	4	-	7
8	52	55.8	29	15	-	-	-	2	-	2
9	63	69.8	44	21	-	-	1	1	-	-
10	52	71.2	37	13	-	-	-	2	4	2
11	30	33.3	10	3	-	-	-	1	-	-
12	48	41.6	20	8	-	-	-	-	-	-
Total	**1567**	**54.4**	**853**	**519**	**5**	**3**	**25**	**39**	**13**	**104**

Five isolates of *Salmonella* spp. were isolated and identified from tap water from different parts of Baghdad governorates depend on morphology, round pale colony with black center on XLD, SS agar. The outcome of biochemical tests clarified that the two isolates of *Salmonella spp*., fermented glucose not lactose appeared as red surface and yellow bottom of KIA slant with gas and H^2O formation for their further conformation API20E and Mini Api 32 were used also in diagnosis according to Jawits *et al.*,(2001) and Amini *et al.*(2010). The serotyping

tests results reflected that all isolates designated as *S. typhimurim* depend on the anti-sera agglutination which done in CPHL.

2- Detection the pathogenesis of the *Salmonella* :- Which includes :- Animal module and the effect of toxins on organs:-

After 5 h from inoculation, the group two of mice were killed for the detection of FA . clinical examinations showed deterioration in health of the

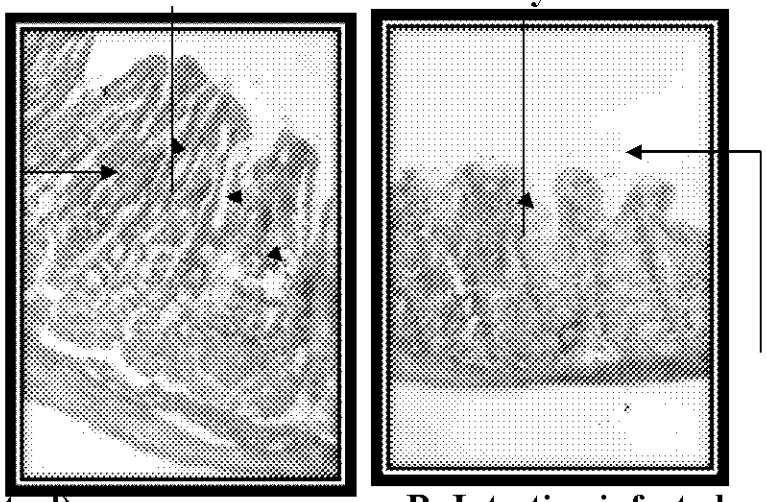
animals with presence of wetness around the anus corrodes, after post – mortem the intestines appeared red and inflated with the liquids this gave positive FA (≥100). After 24 h mice were sacrificed and the pathogenesis effects on different tissues were screened compared with control mice tissues .

Histopathological changes of intestinal section showed slight shorting of intestinal villi with mild inflammatory cells Fig(1 B), when compared to the section of normal group Fig (1A).

Fig (1):- normal structure villi **inflamatory cells villi**

A- Section of normal intestine showing normal stricture villi appearance (x200)(H and E).

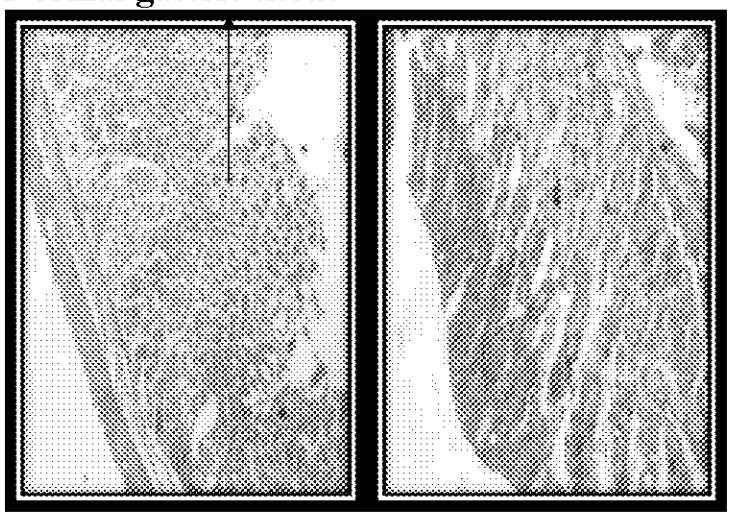
B- Section of intestine showing shortening of intestinal villa with mild chronic inflammatory cells infiltrate.(X200)(H and E).



A- Intestine(control) **B- Intestine infected**.

In other hand Fig(2A) showed normal section of gastric tissue with normal glandular structure appearance of control group , While fig(2B) look like normal appear after 24 h from inoculation.

Normal gastric tissue



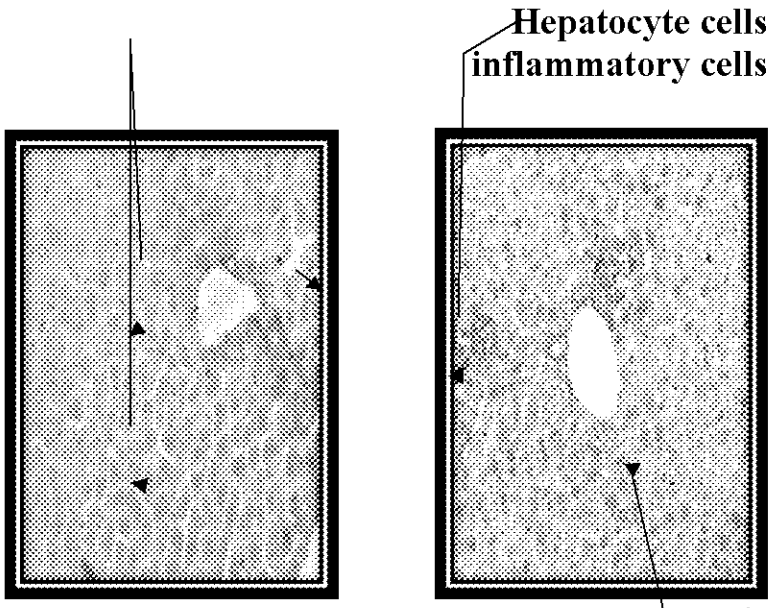
A-Gastric(normal **B-Gastric (infected, look like normal)**

Fig (2):-

A- Section of normal gastric tissue showing glandular normal stricture appearance (x200)(H and E).

B- Look like normalgastric mucosal glandular epithelial (X200)(Hand E).

The investigation results showed that liver suffered from mild degenerative changes, mild inflammatory cells infiltration and necrosis fig(3 B), compared with fig(3 A) which showed the normal texture of liver structure appearance of hepatic cells and central vein.



A-Liver (control) **B-Liver (infected)**

Fig(3):-

A- Section of liver showing normal structure appearance of hepatic cells and central vein .(X200)(H and E).

B- Mild degenerative effect with dispersed necrosis hepatocytes cells with mild inflamatory cells infiltrate. (X200)(H and E).

The renal tissue showed mild inflammatory changes after 24h post-inoculation fig(4B) , compared with control group fig(4A).

renaltubels and glomerular

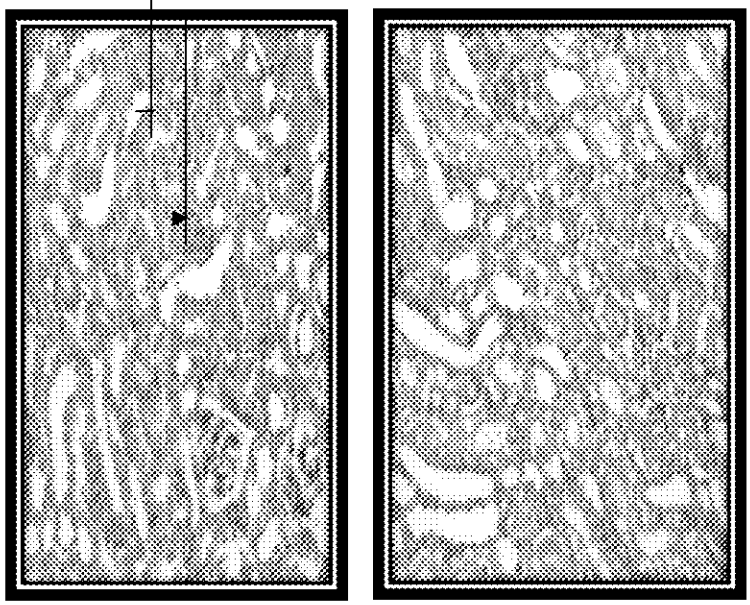


Fig (4):-

A- Normal stricture appearance of renal tissue showing glomerular and renal tubles (proximal convoluted tubuls). .(X200)(H and E).

B- No effects appears on renal tissue.(X200)(H and E).

A-Kidney(control) B-Kidney(infected, look like normal, noeffects)

Salmonella typhimurim most often cause gastroenteritis with watery diarrhea , toxin production can detected in animals models such as sucking mice in which oral inoculation stimulate fluid accumulation. The mechanisms by which stimulate intestinal secretions through intestinal epithelial cells production of proinflamatory cytokines in response to invasiveness. Such as interleukins -8(IL-8) produce and secreted a cross the membrane of epithelial cells after attachment of *S. typhimurim* leading to cyclic AMP level elevated in the in infected intestine (Sears and Kaper ,1996).

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تشخيص امراضية بكتريا *Salmonella* spp. المعزولة من مياه الشرب المجهز لبعض مناطق بغداد في الحيوانات المختبرية

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الخلاصة:-

جمعت 1567 عينة من مياه الشرب المجهز لمعظم مناطق بغداد من بداية الشهر نيسان للعام 2010 ولغاية نهاية كانون الاول من العام 2011. وقد فحصت جميع العينات بكتريولوجيا بالطرائق التقليدية لتشخيص بكتريا القولون الكلية والبرازية والايشريشيا القولونية فضلا عن البكتريا المرضية الاخرى ومنها السالمونيلا، وتم دراسة امراضية خمسة عزلات منها وقابليتها على انتاج الذيفانات من خلال اختبار تجريع الفئران ، واظهرت جميع العزلات البيئية قدرتها على تجميع السوائل بعد 5 ساعات من التجريع وظهور تغيرات نسيجية بعد 24 ساعة من اهمها تلف في بطانة الامعاء, تفاعل التهابي وقصر الزغابات مع ارتشاح في الخلايا الالتهابية, فضلا عن التغيرات النسيجية للكلية والتي ظهرت على شكل تنخر بسيط في الطبقة المخاطية المبطنة للمعدة, في حين اظهرت الخلايا الكبدية تنخر مع ارتشاح واضح للخلايا الالتهابية