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Study of ELISA and antibiotic sensitivity test for *Salmonella enteritidis* as experimental infection in mice

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Abstract

Salmonella enteritidis one of more important as epidemiological bacteria between other salmonella types. It is very important pathologically that cause food poising and gastrointestinal tract infections. This study includes some of immunological changes that appear by ELISA test and antibiotic sensitivity test against these bacteria in mice. ELISA test results appears high immunological response happen after 3 days of inoculation, mean titration readings beginning 0.198 and the maximum mean titration after 15 days of inoculation 1.538 and begin to decrease after this time slowly to remain about 0.297 after 40 days of inoculation. An antibiotics sensitivity test result appears, this bacteria sensitive to Chloramphenicol, Ceftriaxone, Ciprofloxacin and Cotrimaxazol. Resistance to Neomycin, Streptomycin and Rifampicin, while intermediate against Ampicilin and Amoxicillin. Another test we use Vitek system to know bacterial sensitivity against to more another types of antibiotics and to confirm between some of them.

Key words: *Salmonella enteritidis*, ELISA test, antibiotics sensitivity test.

Introduction

Salmonella one of the more causative agent diseases that transfer by food and water. It affects human and animals. Increase food poisoning in word by salmonella one of more problems health that's because food contamination with murins feces and between infected people [1, 2]. Increase cases of food poisoning at resent years due to *S. enteritidis* in poultry that appears healthy birds [3, 4]. It has more than 2200 serotype [5]. *S. enteritidis* non-specific bacteria, affect more of one hosts of animals, laboratory animals and humans [6, 2]. It affect intestine epithelium by invasion M cell and lymphoid tissue of intestine like payer's patches and then distributed to other tissues by blood and lymph [2,7]. It is flagellated bacteria swimming by Run mode or by Tumble mode [8, 9]. Adhesions of *S. enteritidis* with intestinal epithelium it is the first step infection. These adhesions happen by fimbriae on the surface of bacteria [10, 11] it is optimum target to immune system [12, 13]. The bacteria affects all ages of human but it more sever in children, aged and they have chronic diseases. In Iraq this bacteria isolated from dairy product industries cheese and cream [14] water, floor, insects and animal faeces [15]. Human infected by *S. enteritidis* increase in the word from 1980 that cause by egg conception [16, 17]. Chickens affected by theses bacteria in ovary and oviduct or interance bacteria to the egg from the scale [18, 19, 20]. At last 20th years theses bacteria are the main source for infection in poultry fields and cause losses in poultry industry and serious

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problem in human health and poultry [21, 12, 22, 23]. *S. enteritidis* infection in human take food poising characterized by gastroenteritis but some time take sever infection enteric fever and septicemia [24, 25] Salmonella transfer from animal to animal hapent in field, market and slaughter house. Shedding of bacteria increase in more stress factors, transport, crowding and water degrease cause more infection between animals and bird [26,27] animals infection by *S. enteritidis* it take form sub clinically but some of them still carrier cows suffer from fever, diarrhea, some time abortion and endometritis. Calf arthritis high mortality and sever diarrhea [28, 29].

Material and methods

Laboratory animals

One hundred white balb/c mice 15-20gms weight divided to cages 6 mice for each cage.

Ag preparation

Bacterial isolation on SSagar plates and incubated at 37c° for 24 hrs and then harvested bacterial culture and washing by PBS for three times respectively by centrifuge at 3000 c/min for five minutes for each once. Freezing and thawing for several times and centrifuging to take supernatant that contain soluble Ag and incubated in -20c° at using [30]. Determination protein concentration of Ag depend on [31] protein combination with collar agent pirogallolo Red-Molibdato in acidic solution to compound collar complex that resemble with protein concentration. Mixed component in circular system vortex and incubate at 37c° for 5minits. Mixed again and read extinction against blank by spectrophotometer at wave length 600 nm and application this formula

$$\text{Total protein liquor (mg/dl)} = \frac{E\ \text{sample}}{ESTD} \times C.STD$$

E=Extinction
C.STD=standard conc. 50mg/dl

Serum Preparation

Serum prepared from blood sampling of mice that used in test as this table of time

Day	Day	Day	Day	Day	Day	Day	Day	Day	Day	Day
3	6	9	12	15	18	21	24	27	30	40

The serum persevered in -20c°

Check board titration

For estimate optimum concentration of Ag that recognize positive and negative results and optimum dilution of serum used titration by several concentration of Ag

1:40	1:80	1:160	1:320	1:640

And several dilution of serum positive and negative control

1:100	1:200	1:300	1:400	1:500

And determination optimum concentration of Ag and serum dilution by test best reading that differentiate between positive and negative.

Positive control
Serum of 10 infected mice presented by intraperitoneal inoculation of *s. enteritidis* and give positive results of bacterial isolation from these mice in blood culture.

Negative control
Serum of 10 non infected mice presented and give negative result in blood culture.
Cut of point determent by mean optical density (OD) of negative control added to multiply of standard deviation [32].

Procedure
200µl of Ag about 1.5µg/well after dilution 1:40 with coating buffer for each well of micro plate titration board, covering and incubated at 4c° to night. Wash plate with washing buffer four times dried with drying paper. Put 200µl of blocking buffer and incubated at 4c° 24hrs washing four times and put 200µl of diluting serum 200:1 with diluting buffer for each well of plate, incubated for one hour at 37c°. Wash five times and adding 100µl of diluting conjugate 1000:1 for each well and incubate for one hour at 37c°. Washing and adding 100µl of substrate for each well of plate and putting in dark place at room temperature. Stopped activation by adding 50µl of HCL. Result reading by ELISA reader spectrophotometer at wave length 450nm.

***Salmonella enteritidis* antibiotic sensitivity test**
Depend on [33] and test nine types of different antibiotic with *S. enteritidis* type D by using specific discs.

Method
Prepared bacterial suspension with normal saline and cultured on Muller Hinton agar with equal distribution. Discs distributed at five discs for each plate, incubated at 37c° for 24hrs. Measuring antibiotic sensitivity depending standard tables. Also we depend on BioMerieux Vitek system that is important in identification and antimicrobial susceptibility testing and for rapid results.

Results
Depended on ELISA test to determent a level of immune response against *S. enteritidis* at limited time table. The results estimate the best serum dilution that differentiate between negative and positive is 1:200, the optimum antigen concentration is 1:40 and conjugate dilution is 1:1000 mean of OD negative control 0.22 and standard deviation 0.02 that the cut of point for reader 0.26 . The maximum, minimum and means OD of serum samples at days for each sample as in table (1).

Table (1) maximum, minimum and means OD of ELISA test for each case at determent time

Group	Days after injection	Maximum	Minimum	Means
1	3	0.213	0.185	0.198
2	6	0.297	0.220	o.256
3	9	0.634	0.542	0.595
4	12	1.012	0.924	0.972
5	15	1.643	1.430	1.538
6	18	1.580	0.972	1.319
7	21	1.607	0.952	0.853
8	24	0.956	0.210	0.535
9	27	0.665	0.210	0.438
10	30	0.453	0.185	0.307
11	40	0.439	0.210	0.297

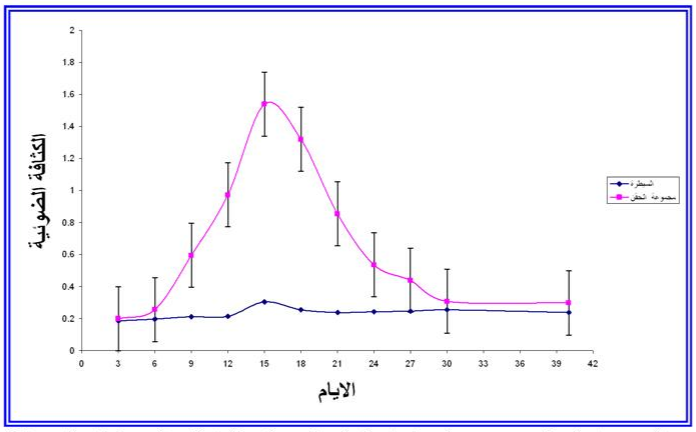

Figure (1) optical density distribution for infected mice serum by intraperitoneal rote

Table (2) type, concentration and sensitivity of *S. entritidis* for some antibiotics

No	Antibiotic	Symbol	Concentration	Type of resistant
1	Ampicillin	Am	10mg	I
2	Chloramphenicol	C	30mg	S
3	Neomycin	NE30	30mg	R
4	Streptomycin	S10	10mg	R
5	Ceftriaxone	CRO30	30mg	S
6	Ciprofloxacin	CIP5	5mg	S
7	Rifampicin	RP5	5mg	R
8	Cotrimoxazole	TS25	25mg	S
9	Amoxicillin	A25	25mg	I

Disc: mast group LTd. Company UK
R: Resist
S: Sensitive
I: intermediate

Table (3) sensitivity test of *S. entritidis* for some of antibiotic by using BioMerieux Vitek system

No	Antibiotic	Mic µg/ml	Sensitivity
1	Ampicillin	1	S
2	Aztreonam	8≥	S
3	Cefazolin	8 ≥	S
4	Cefepime	4≥	S
5	Cefotetan	16≥	S
6	Ceftazidime	8≥	S
7	Ceftriaxone	8≥	S
8	Ciprofloxacin	1	S
9	Gentamycin	4	S
10	Imipenem	4≥	S
11	Levofloxacin	2	S
12	Piperacillin / tazobadem	8≥	S
13	Tobramycin	4	S
14	Trmethsulf	10≥	s

Mic = minimum inhibition concentration

Discussion

Antimicrobial resistant against salmonella important to know the development of this resistant and distribution and how to control of this bacteria by drug selecting and optimum dose [34,35] this results appear multi resistance for several antibiotic that use in hospitals and appear sensitive for others. The cause of this multi resistance is trance from resistance bacteria to sensitive that alive in same environments by genes on conjugated plasmid [36]. Some of this bacterial resistance related with R-factor that lead to trance multiple resistance of antibiotic at some time and that happens mostly in bacteria that cause diarrhoea [37,38,39]. Adding antibiotic randomly to animals feed to growth stimulation and increase production cause bacterial resistance in many countries [40,41] now most of salmonella species resistance to many antibiotic and cause sever disease in AIDS patients or in organs transplantation [7].

Used white mice in this research because it sensitive to infection and give high immune response. Depend intra peritoneal rout of injection to wide spread bacterial dissemination to different organs and used ELISA test because it is high sensitive and high specific [42]. Alive bacteria have high stimulation with one dose and have whole antigens and have ability to multiplication and toxins production to attack different body tissues [43]. Increase Abs titters due to T helper lymphocytes stimulation and production sensitive plasmid cells and memory lymphocytes.

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دراسة اختبار الاليزا وفحص الحساسية الدوائية للسالمونيلا المعوية كأصابة تجريبية في الفئران

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الخلاصة:

جرثومة *Salmonella enteritidis* واحدة من أهم الجراثيم الوبائية من بين أنواع السالمونيلا الأخرى و مهمة جدا" من الناحية المرضية لأنها تسبب التسمم الغذائي وإصابات القناة المعدية المعوية. وهذا البحث تضمن دراسة بعض التغيرات والاستجابة المناعية للفئران بعد حقنها بهذه الجرثومة والتي تم قياسها باختبار الأليزا كما وتم فحص الحساسية لبعض من المضادات الحياتية ضد هذه الجرثومة. وأظهرت نتائج اختبار الأليزا حدوث استجابة مناعية واضحة بعد 3 أيام من الحقن ومعدل القراءات 0،198 وأعلى معدل للقراءات كان بعد 15 يوم من الحقن وكان 1،538 وهذا المعدل بدأ بالنقصان بعد هذا الوقت وبشكل بطيء ليكون حوالي 0،297 بعد 40 يوم من الحقن. كما وأظهرت نتائج فحص الحساسية للمضادات الحياتية بأن هذه الجرثومة حساسة إلى كل من كلورمفنيكول وسفترياكسون وسبايروفلوكساسين وكوتريماكسازول. وكانت مقاومة إلى كل من نيومايسين وستربتومايسين وريفامبسين. بينما كانت متوسطة الحساسية لكل من امبسيلين واموكسيسيلين. ألاختبار الآخر تم استخدام نظام فايتك لمعرفة حساسية الجرثومة تجاه أنواع أخرى من المضادات الحياتية وللمقارنة مع بعضها.

الكلمات المفتاحية: السالمونيلا المعوية ، اختبار الاليزا، فحص الحساسية.