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RESEARCH ARTICLE

A study on antibiotic sensitivity, bacterial dissemination and humoral immunity against experimental infection of white mice with Salmonella enteridis

Khalil H. Al-Jeboori[1], Laheeb J. Al-Hashimi[1] and Haitham I. Baqir[2]

1. Dept. of Pathology, College of Veterinary Medicine, Univ. of Baghdad, Iraq.
2. Scientific Researcher, Central Public Health Laboratory, Ministry of Health, Iraq.

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Corresponding Author

Khalil H. Al-Jeboori

Abstract

A local strain of Salmonella enteridisobtained from the public health laboratory, the bacterial strain reidentified to be sure S. enteridisusing cultural, biochemical test, API 20 E and slide agglutination test by Vitek test. The LD50 of this microbe was $1.4*10^6$ CFU. This bacterial agent was sensitive to chloramphenicol, cefriaxone, ciprofloxacin,cotrimoxazole and resistant to neomycin, streptomycin and rifampicin and variable sensitivity to amoxicillin and ampicillin. S. enteridiswere disseminated into all over the organs and persisted for 2 weeks in all organs except that in liver and spleen it persisted for 27 days post inoculation. Good immune response initiated following S. enteridisexperimental infection in white mice detected by Elisa test.

Conclusion:

1. S. enteridiswas sensitive to the most antibiotic and resistant to some one.
2. S. enteridisdisseminated all over the body organs of white mice.
3. S. enteridisinduce a good humoral immune response.

Introduction

Infections with Salmonella enteridisconsider the main reason of food poisoning in man causing Gastroenteritis and infect other anmals and avian species including different pathological disorder (1). In certain cases these microbial agents colonize the avian intestine as in apparent carriers and during fecal excretions, it transmitted to other birds and meat contamination occurred (1, 2). S. enteridisconsidered the intra and extra – cellular invasive pathogen pass through ileum epithelia into peyer's patches and through blood or lymphatic vessels reach into other organs (3). Murine salmonellosis is a common disease caused by S. enteridisand S. typhimurium, associated with sever enteritis and septicemia in other internal organs (4). For the importance of S. enteridisin human and animals causing different pathological disorders; this study aimed to identify antibiotic sensitivity, bacterial dissemination and humoral immunity following experimental infection of white mice with S. enteridis.

Material and Methods

A local strain of Salmonella enteridisobtained from the public health laboratory center reidentified again to confirm the diagnosis S. enteridis using biochemical tests (5), API 20 E (BioMerieux, CFrance), slide agglutination test using Vitek instrument (BioMerieux) and the standard immunological sera already prepared (BioMerieux and Pasteure, France and Mast, England). The tests were done according to (6) method.

Antibiotic sensitivity test:

Using (nine types of antibiotics) Ampicillin, Chloramphenicol, Neomycin, Streptomycin, Cefriaxone, Ciprofloxacin, Rifamicin,Cotrimoxazole and amoxicillin according to (7).

Determination of the lethal dose-50:

LD-50 dose of S. typhimuriumwere $1.4*10^6$ CFU according to (8) method. Then 0.25ml ($1.4*10^6$ CFU) of bacterial suspension were injected I/P in 33 white mice and 0.25ml of phosphate buffer saline were injected I/P in (11 white mice) control group. 3 mice sacrificed every 3 days intervals and bacterial isolation were done from the sacrificed mice organs. Blood samples were taken from sacrificed animals.

Immunological tests:

All the blood samples were taken from the sacrificed mice and the serum samples were kept at 20 C° until the using time. The enzyme linked immunosorbant assay (Elisa) was done according to (9) technique using Micro Elisa reader (sky line ELMI Beckman counter AD340). The results of the tests sera were read by Elisa reader spectrophotometer under 450 nm wave length.

The Results and Discussion

The results indicated that all the cultural characters, biochemical tests API 20 E test and slide agglutination test, that this strain is reidentified as Salmonella enteridistype D. These tests were identify sure for the diagnosis of S. enteridisin the world (10). The LD50 of this strain was $1.4*10^6$ CFU.

Antimicrobial susceptibility testing:

S. enteridiswas sensitive to chloramphenicol, cefriaxone, ciprofloxacin, cotrimoxazole and resistant to neomycin, streptomycin and rifamicin, whereas, variable results against amoxicillin and amicillin. Also S. enteridiswas sensitive to all antibiotics by BioMerieuxVitek instrument under minor inhibitory concentration.

The resistance against antibiotics occurred through the transmition of bacterial plasmids between the different Gram negative bacterial species which occurred in the intestine (11). S. enteridisstrain which used in this study was resistant to some antibiotics and sensitive to others, this resistance may related to transmition of resistance feature from resistant strain to sensitive strain which present together, through genes carried on conjugated plasmids responsible for this resistance feature and pathogenesis factors (12). Also some resistance features of the bacteria was belong to the R-factor through which the multiple resistance against the antibiotics (13). The different antibiotic resistant strains occurred in human when abuse of the antibiotics in treatment of some diseases under no physician prescription and or using some antibiotics with few doses, for this reason antibiotic sensitivity test was important (14). Also the addition of some antibiotics randomly animal rations induces similar antibiotic resistant strains (15).

The dissemination of S. enteridisin the internal organs of mice following I/P inoculation:

During forty days of S. enteridis I/P inoculation in mice, the microbial agents were dessimated in the all internal organs of mice**(Table – 1),**the microbial were persisted in liver and spleen for 27^{th} days post inoculation. It persisted in heart for 18^{th} days whereas; it persisted in mediastinal lymph node, lungs, kidney, intestine and periton for 15^{th} days post inoculation and in brain for 12^{th} days. Following inoculation of mice with S. enteridis, some mice were died at the next day of inoculation due to endotoxemia (16). Whereas in the living animals the microbial agent was gradually proliferated in the periton and surrounded tissues (mediastinal lymph node & pancreas) then through the thoracic duct reach into the heart and into other organs in which the microbial agents was persisted for 27^{th} days post inoculation such as in liver and in spleen. Similar findings were reported by (17) and with other salmonella species such as for S. typhimurium(18) and for S. typhi(19) and for S. paratyphiA, B (20, 21). All those workers reported that the dissemination of salmonella species in these organs due to their enriched with reticulo endothelial tissue.

Table – 1: The dissemination of S. enteridisin the mice organs following I/P experimental infection

Periton	Brain	Intestine	Kidney	Lung	Mediastinal lymph node	Heart	Spleen	Liver	Post inoculation days
+	+	+	+	+	+	+	+	+	3 days
+	+	+	+	+	+	+	+	+	6 days
+	+	+	+	+	+	+	+	+	9 days
+	-	+	+	+	+	+	+	+	12 days
+	-	+	+	+	+	+	+	+	15 days
-	-	-	-	-	-	+	+	+	18 days
-	-	-	-	-	-	+	+	+	21 days
-	-	-	-	-	-	-	+	+	24 days
-	-	-	-	-	-	-	+	+	27 days
-	-	-	-	-	-	-	-	-	30 days

-	-	-	-	-	-	-	-	-	40 days

No. of animals (33), No. of sacrificed animals (3), Dose ($1.4*10^6$ CFU/mice), (+) positive, (-) Negative.

Humoral immune response against S. enteridis(Elisa Test):

The result of Elisa indicated that the proper dilutions for animal sera were 1:200 and for S. enteridisAgs were 1:40 and cutoff value 0.26 and all the optical values above 0.26 were considered positive **(Table – 2).**

Table – 2: Lower, upper and mean values of optical density for Elisa during experimental infection.

Mean values	Lower value	Upper value	Days post injection	Group
0.198	0.185	0.213	3	1
0.256	0.220	0.297	6	2
0.595	0.542	0.634	9	3
0.972	0.924	1.012	12	4
1.538	1.430	1.643	15	5
1.319	0.972	0.580	18	6
0.853	0.952	1.607	21	7
0.535	0.210	0.956	24	8
0.438	0.210	0.665	27	9
0.307	0.185	0.453	30	10
0.297	0.210	0.439	40	11

Elisa test used in this study gave high sensitivity and specificity comparable to other test (22). Good immunological reaction occurred in microtiter plate Elisa with best Ag dilution (1:40) which characterized between positive and control state using Horse radish peroxidase (HRP) conjugate (1:1000) and gave proper readings (optical density) after addition the substrate. All the positive sera occurred in the animals infected with the S. enteridis,in addition totheir Ags enhance the good humoral immune response detected by Elisa in this study. The living organisms (S. enteridis) used in this study instead of killed organisms because the killed organisms need multiple doses to enhance immune response whereas, living organisms need only single dose to enhance the immune response in addition to that living organisms contain all the Ag comparable to killed organisms and the living organisms proliferate continuously and supplied the mice body by recurrent Ags since good immune response (23).

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