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Protective Immunity against Virulence Uropathogenic Escherichia Coli in Mice

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Abstract

Infection in the urine tract is more dominate as a medical condition around the world in the hospital and society, especially from young women and pregnant women. The reason for disease the first is uropathogenicEscherichia coli, which has several agents that help in the development that disease in addition to its the capability to stick on a host epithelial cell in urine tract with an assembly the biofilm, which is the most important factor for the disease. Therefore, a vaccine was made for these local isolates to avoid recurrent urinary tract infection and to obtain protective immunity by a laboratory mouse model. After isolation and diagnosis of 90 positive isolates using chrome agardiagnostic from women with urinary tract infection from hospitals in middle Iraq to Wasit province for the period from Jan. 2019 to Apr. 2019. All isolates were tested to detect virulence factors. One isolate was chosen for the determine LD50, immunization and vaccine model, then to estimate the antibody (IgG) level during immunization in the serum of mice at 6 weeks. Results proved that isolates showed positive (71.11%) biofilm, (23.33%) α-heamolysin, (31.11%) serum resistance, and (41.11%) MRHA and (5.55%) MSHA , while LD50=($1*10^{10}$).There are highly significant differences when using isolate in the level of IgG reaches the high peak ranged (749.1 ±20.4) in 6 weeks as compared with control. The results showed that the local vaccine for this isolation provided preventive protection by inducing a protective immune response in mice to protect against infection.

Keywords: *humoral immune response,E.coli, Virulence factors, LD50.*

Introduction

When uropathogenicE. coli (UPEC) have the capability to step inside the host urine tract with reaches to 10^6 colonies per ml urinary tract infection occurs, and As a result of the infection, several diseases may develop, including acute, chronic, recurrent and bacteriuria infection [(1)].After a period of urinary tract infection, the bacteria begin to enter the latency state, which will be the future and express recurrent urinary tract infection resulting from the reactivation of the bacteria to have factors that help it in addition to that the lack of correct diagnosis of this infection will expose the patient to a serious medical condition[(2)]. UPEC characterized by the possession of several virulent factors have a close role in the continuation of colonization and the occurrence of the pathogenesis of the urinary tract, and the biggest remarkable agents are biofilm, hemolysin, and fimbriae[(3)].Fimbriae There are two types according to immune classification through special receptors which are mannose resistance hemagglutination (MRHA) and mannose sensitive (MSHA[(4)]. The multiple virulence factors of the E. coli strains shown during the urinary tract infection are dangerous to the patients' lives. It is therefore necessary to develop a protective immune response by a vaccine against these bacteria and their virulent agents [(5)]. Therefore, the research aims to provide a suitable vaccine for local isolates from hospitals in middle Iraq to the E. coli bacteria which have harmful factors and reduce the cost of importing ineffective vaccines and avoid the side effects of antibiotics for pregnant women. .

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Materials and Method

Collect and diagnose bacteria:

Period from Jan. 2019. Until Apr. 2019 Urine samples were collected form women with urinary tract infection at AlAziziyah, AlEssaouira, and Al Karama hospitals with about 205 samples. Following the method [6] all isolates were obtained using conventional tests and confirmed by using(Chrome-Agar ™ Orientation / France) diagnostic for E. coli. [7].

Detection of virulence factors for E.coli:

Phenotypic detection for 90 E.coliisolates from urine was done to detect the ability of Hemolysin production,Hemagglutinationmethod, and biofilm formation[8], Serum resistance by [9].

Preparation of the vaccine for virulent bacteria:

The most ferocious and positive local isolates were selected for all virulence factors using the main method for prepare vaccine which depends on [10].

Immunization schedule:

White mice weighing 25 grams aged 62 to 96 days were purchased from the University of Baghdad College of Science / Animal Branch and under microbial-free conditions and pressed in cages for Feeding for 5 days without giving them fat in food and then placed under control in cages and divided into three groups for the purpose of giving the vaccine prepared in advance

1- The first and the second group dose were 0.5 ml of colony $1x10^8$cfu/ml injects subcutaneously twice in two weeks.

2-The third group (control) was given the solution in the same manner and a dose of 0.5ml from PBS pH = 7.2±2

Where the serum was collected during the immunization period from 2 to 6 weeks at the end of each immunization time and stored at -18C° until used in the verification of the quantity of antibody [11]. .

Estimated LD50 and challenge test:

According to the following equation, the lethal dose 50 was estimated by counting dead and live mice in each group for one month, which was injected subcutaneously with bacteria Choose most vicious local isolate E. coli. The live bacterium takes to make gradient dilution from 10^{-1} to 10^{-8} according to [12]. Also, lethal dose 50 worked dependent on the method mention in [13]. 48 normal animal separate to 8 cages , then groups injected by the subcutaneous method with use 0.5ml of diluents except one group the control that gives 0.5 ml from PBS pH=7.2±2 by the same method for challenge test ,totally cages watch forone month to detect lethal and survival mice. .

$$\% \text{ of Mortality} = \frac{\text{Total Dead}}{\text{Sum of (Total live + Total Dead)}}$$

Determination (IgG) quantification in mice serum during immunization:

From 2 to 6 weeks of administering the vaccine, then was used (Mouse IgG Elisa kit) to quantify of antibody, and to investigate humoral immune response in mice during immunization.

Data Analysis

Program IBM spss ver.22 Used to analysis data to obtained significant different between groups of mice

Results and Discussion

Isolation and Diagnosis:

In our examination from 1/1/2019 until 30/4/2019, an aggregate of 200 urine tests was gotten and refined. Out of 205 samples of urine, results demonstrated 140 (68.29 %) were positive culture and 45 (21.95 %) were negative culture while 20 (9.75%) were indicated contaminate urine culture Table (1) . The conveyance of confined relies upon the age of (20 to 62) years old, for female patients, the age went from (20 to 29) was 40 (28.75%) and this is the most overwhelming ages contaminated with gram-negative microorganisms. The age were Showed in (Table 2)

Table 1. The Distribution percentage of isolated depends on age from (20 to 62) years old.

Age group (years)	No. of isolating	%
20 - 29	40	28.75
30 – 39	24	17.14
40 – 49	21	15
50 – 59	26	18.57
60 – 62	29	20.7
Total of isolate	140	100

Table 2. Results of the biochemical test and CHROM-agar.

Bacteria	Biochemical Test						
	%	Gram stain	Indole	Citrate	Urease	Motility	Color on Chrom–agar
E. coli	64.28	G-ve	+	—	—	+	Dark- violet
K. pneumonia	19.28	G-ve	—	+	+	—	Metallic- blue
P. mirabilis	9.28	G-ve	—	—	+	+	Brown

Results showed the percentages of all isolates taken from urine using biochemical tests and chrome agar was as follows: E. coli 64.28%, Klebsiella spp. 19.28%, and P. mirabilis 9.28%. In another study, the highest percentage of our study results was about 83.3% [14]. in the (Table 3). Therefore, the results appeared E.coli dominates in infection of urine tract. Showed in (Fig.1) color of isolates on chrome- agar

Figure1. (A)E.coliDark- violet, (B)K. Pneumonia Metallic -blue ,and(C) P. mirabilisBrown.

Virulence factors:

The result of our study to detect Heamolysin produce by 21(23.33%) isolate ofE.coli from UTI that identical to outcome obtained by [15]. α- hemolysis target erythrocyte of patient, also, cause an effect on it to lead inflaming, damage, and weak patient resist. Also provoke Release Superoxide, H2O2 and O2 consuming by cells renal, consist of sensitizing by immune cell [16].Out of the total 90 isolates we tested 37 isolate shows Mannose resistance hemagglutination as percent (41.11%) and mannose sensitive hemagglutination in percent (05.55%) this result very close to result of the study done in India [17]. While inconsistent with another study included low percent in (MRHA) Mannose resistance hemagglutination (28.09%) and high percent in (MSHA) mannose sensitive hemagglutination (8.5%) [18]. Hemagglutination is mediated by fimbriae. The ferocity is determined for UPEC type 1- fimbriae connection with mannose element including of receptor on host cell in a urinary tract, and play important role in first step of biofilm by attachment to the epithelial cell

of a host [19].In our study, a total of 90 isolates detected to biofilm formation, 64 isolates Strong biofilm produce was (71.11%), percent of moderate biofilm produce was 18 (20%), and weak biofilm produce was 8 (8.88%) that result agreement with study conducted in West Africa [20]. However, our results differ from the results of the study carried out by [21]that showed (45%) weak biofilm produce, (49%) moderate biofilm produce, (5%) strong biofilm produce.The present study result about virulence factor Serum resistance showed 28 isolates ofE.coli in percent (31.11%) positive that are in close with the results of the study achieved by [22]. As compared with another study [23] the result was a higher (90%). Serum of human reluctance by E.coli is a critical ferocity factor . All outcomes showed in (Table3) with (Fig.2)..

Table 3. Percentage of ferocity agents for E.coli from urine.

V. Factor Result	Heamolysin	Biofilm	MRHA	MSHA	Serum resistance
Positive	21(23.33%)	S 64 (71.11%) M 18(20%) W 8 (8.88%)	37(41.11%)	5(5.55%)	28(31.11%)
Negative	78(86.66%)	0 (0%)	53(58.88%)	85(94.44%)	62(68.88%)
Total	90(100%)	90(100%)	90(100%)	90(100%)	90 (100%)

S=Strong biofilm, M=Moderate, W=Weak.

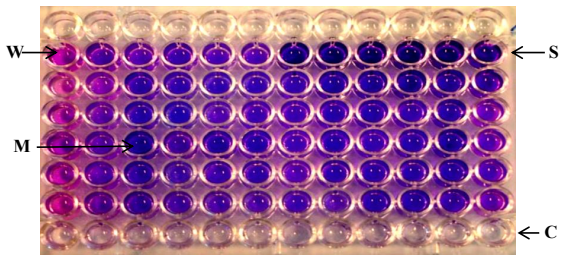

Figure2 . biofilm forming test by microtiter plate (U shape)

(S)Strong biofilm, (M) Moderate, (W) Weak, (C) Control

LD50:

According to the dose used to calculate the lethal dose 50 (1X1010) as show in (Table 6) The experimental challenge test was performed for all groups in intraperitoneally injection 4 LD50 (4 X 1010) method for the virulent E. coli. The results showed that the fortified mice showed mild signs within 2 days and return to normal within a week. The control group showed death within 3 days our result came very close to study (24), and In this search discover the prepared vaccine (whole-cell bacteria) protected animal (mice) against infection by the intraperitoneal route, In a above study showed incompatibility immunization was used in several ways where the mice were fortified by the intraperitoneally but did not survive whenusing the intranasal method. Where the survival rate of the protected mice was better than the control group, UPEC bacteria its first reason in infection of urine tract in a host. In our experiment, we tested the vaccine susceptibility to provide protection against severe isolates and explained that immunized mice are better to survive compared to the controlgroup. Table (4).

Table 4. Calculation of the lethal dose 50 of E. coli in mice

Group	Dose	Live	Dead	Total live	Total dead	%
1	1x1013	0	6	0	21	100
2	1x1012	0	6	0	15	100
3	1x1011	2	4	2	9	81
4	1x1010	3	3	5	5	50
5	1x109	4	2	9	2	18
6	1x108	6	0	15	0	0
7	1x107	6	0	21	0	0
Control	PBS	6	-	-	-	0

Determine IgG quantity:

The results reported in a (Table5) to detect Antibody quantity with a mean was (192±11.2) at zero weeks from the injection. Then reaches to the middle value of mean (405.3 ±16.1) in 2 weeks .then reach to a high value at 4 weeks (749.1 ±20.4) The result of values showed a significant increase of IgG quantity at the p-value of least of (0.05 value) from 2 to 6 weeks when compared with the control group,In general, the UPEC strain contains several virulence factors, so the vaccine used from this local strain must capable of stimulating humoral immunity representing by antibody(IgG)antagonistic effect to virulent agents , which appear at varying phases of urine tract infection.So the local vaccine is promising and efficient to protect against virulence factor of UropathogenicE.coli and infection, as mentioned in a study [25].

Table 5. Antibody (IgG) quantity in serum of mice by Elisa during 6 weeks

Treatment (weeks)	**Immunized group with UPEC vaccine Mean ± SE**	**Control group Mean ± SE**
0	192±11.2 A	195.9 ±8.3 A
2	405.3 ±16.1 A	202 ±12.3 B
4	749.1 ±20.4 A	188.9 ±11.9 B
6	660 ±22 A	190 ±10.9 B

Conclusion

After assessment virulence factors for all isolate then using fierce local isolation from 90 isolate which that gave protective immunity against virulence factors and infection of UPEC in immunized mice compared with control mice with use an important parameter is determined the quantity of antibodies (IgG) in the mice serum during immunization 6 weeks to monitor and investigate the vaccine activity to stimulate the humoral immune response, and immune system. .

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Conflict of Interest: Non

Source of Findings: Non

Ethical Clearance: Non

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