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RESEARCH ARTICLE

Immunization against Multi drug Resistance Uropathogenic *E. coli* Isolate from Urinary Tract Infection in Pregnancy

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ABSTRACT:
Uropathogenic *Escherichia coli* (UPEC) is characterized by complex natures with ceaselessly developing for virulent from non- virulent isolates cause strong illness. Vaccines against UPEC are not widely available, leading to exceeding the incidence of these isolates impervious for the antimicrobial agent. This requires constant efforts to control diseases and to know the development of infection and immune response. After infection and immunization, ninety clinical isolates identified as UPEC were obtained from hospitalized patients from three hospitals in south Iraq and identified by the biochemical test. All isolates were tested for susceptibility testing. One isolate was chosen for the immunization and vaccine model, the one remittent for most antibiotics. ELISA was used to assessments of immunoglobulin (IgG) level to serum mouse at 14, 28 and 42 days of immunization. Results proved that isolate showed resistance to all antibiotics. There are highly significant differences when using UPEC in the level of IgG reaches the peak ranged (685.3 ±49.8) at 28 days as compared with control for assessment humoral immune response. Also, high significant differences thickness of the footpad skin reaches (5.021±0.210) after 24 hours of the immunized mice, so that vaccine with this isolate has a potential for development as a candidate vaccine for protection against infection.

KEYWORDS: Immunization, Vaccine, UPEC, MDR, IgG.

INTRODUCTION:
The most common type of infection is urinary tract infection, which is exposed to women during her life, especially during pregnancy, which leads to the aggravation of the condition and responsible for this is the UPEC bacteria[1,2,3.] The adhesion step to the epithelial cells in the urinary tract is important, enabling bacteria to interfere with receptors on the surface of the host cells by secondary structures such as fimbriae, which represent critical virulence factors in UPEC[4.] Uterine epithelial cells represent a physical defense line against the pathogens as well as the release of biological material defenses as part of the immune response in the patient's body[5]. Immunoglobulin plays an important role in the elimination of bacteria and facilitates compatibility and association with macrophage as a defensive function[6].

One of the most related works of well-known methods of stimulating immune response and immune cells is the vaccine to overcome chronic diseases caused by this bacterium[7.] UPEC is the most common and important bacteria, with the increasing problem of resistance to antibiotics, the development of immune therapies is important, one of the most successful methods in medicine for the treatment of infectious diseases is the use of a vaccine. Adaptive immune response to UPEC will protect the bladder from reinfection and the whole urinary tract. The vaccine protects women aged 20-50 years from recurrent urinary tract infection. Also, intraperitoneally and intravesical protection against the rising urinary tract infection is provided by an immune response using a vaccine of this type of bacteria killed[8.]

As compared with the above studies, this paper specifically investigates immunization in contradiction of multidrug resistance Uropathogenic *E. coli* isolate from urinary tract infection in pregnancy. In addition to the Introduction Section, this paper includes Material and Methods, Tests, Outcomes, Discussion and Conclusion as depicted useful parts of this study.

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MATERIAL AND METHODS:

Isolation and Diagnosis *E. coli*:

This bacterium has isolated the duration between Sep. 2018 to Dec. 2018 from three hospitals and samples were obtained from women patients have UTIs, add up to 205 midstream sample tests gathered from the patients associated with urinary tract contaminations were refined by the semi-quantitative culture method[9]. All isolates from the UTI were distinguished by biochemical tests were carried out and served as the confirmatory test of *E.coli* isolates examined on CHRO Magar™ Orientation media[10].

Antibiotics Test:

Antibacterial agents test were carried out through the disc diffusion method plate dissemination strategy utilizing Muller-Hinton agar media (Himedia, India) The segregates were screened for defenselessness to the antibacterial agents: Ciprofloxacin (5µg) (CIP), Amikacin (30µg) (AK), Nitrofurantoin (300µg) (NIT), Trimethoprim-Sulfamethoxazole (1.25/23.75µg) (STX), Gentamicin (10µg) (GEN), Piperacillin (100µg) (PIP), Nalidixic corrosive (30µg) (NA), Tetracycline (30µg) (TE), Imipenem (10µg) (IPM), Ceftriaxone (30µg) (CRO), and Cefotaxime (10µg) (CTX) (Bioanalysis, Turkey)[11]. A bacterial suspension was set up by getting 1-2 states from unadulterated plates into 2.5ml of sterile refined water. The solution was poured and spread on the surface of agar with the use of sterile swabs in various ways. Antibacterial plates were put onto the medium surface by a sterile combine of forceps. Then overnight for plates by incubation at 35C° to 18-24hr. in an oxygen-consuming climate; at that point restraint zones around the antibacterial agent were estimated[12].

Preparation of UP*E. coli* Vaccine:

To prepare a local vaccine against *E. coli* Iraqi isolate from urine selected is multidrug isolate used in this study vaccine was prepared in a modified manner from the original method; the cultured bacteria were cultured in BHI broth at 37°C to 18-20 hrs. And collected by solution PBS pH=7.4 then washed 3 times in the same solution by centrifugation at 600 X g for 15 min. at 4°C and resuspended in PBS solution with Colony number of *E.coli* equal to $1x10^{8}$ cfu/ml. Finally, a step used temperature 60°C to kill *E.coli* in the solution for one hr.[13]. before using prepared vaccine experiment with purity and sterile according to[14].

Mice fortification schedule:

Male BALB/C mice weight were ranged from 20-25 grams, and their age ranged between 6 to 8 weeks obtained from the National Center for Cancer Research and housed under specific pathogen-free conditions that were breeding in different cages of mice place at the Technical Institute Kut. The experiment animal separates into three groups. The first group fortification by the subcutaneous method with prepared vaccine 2 times in 14 days a dose of 0.5ml with colony $1x10^{8}$ cfu/ml. The second group was injected by subcutaneous with 0.5ml of PBS. Serum was collected from 14, 28 and 42 days post injection with booster fortification dose. Serum was stored in -20ºC until use with ELISA[15].

Challenge test:

Choose most resistance local isolate *E. coli* to challenge. The live bacterium has gradient dilution from 10^{-1} to 10^{-8} according to[16]. Also, lethal dose 50 worked dependent on the method mention in[17]. 48 normal animal separate to 8 cages all groups injected by the subcutaneous method with use 0.5ml of diluents except one group the control that gives 0.5ml of PBS, pH=7.4 by the same method. Cages are watched for one month to detect lethal and survival mice.

Assessment IgG levels in serum:

Post-immunization the immunity was detected by Elisa. All reagents and were prepared according to the manufacturer (Mouse IgG ELISA Kit ab157719). That experiment utilizes to detect immunity type humoral with an assessment of antibody during fortified in 14, 28 and 42 days.

Delayed-type hypersensitivity test:

This test worked after the third week from immunization, first of all, inject the antigen of bacteria that prepared by the method[18] finally, measured the thickness of the skin by venire caliper before and after injection dependent on the technique[19].

Data analysis:

For analysis of our data to reach different value among diverse groups used program SPSS version 21.

Outcome and Discussion:

Table 1 shows the Percentage of bacteria by using the CHRO Magar™ Orientation and conventional biochemical tests as following the Firstly *E. coli* in percentage (64.28%), secondly *Klebsiella* spp. in percent (19.28%), thirdly *Proteus mirabilis* in percent (9.28 %) and Fourthly *Pseudomonas* spp. In percentage (3.57%) respectively, and the gradation of isolates was consistent with the study[20] were the most prevalent bacteria isolated in that study.

Table 1. Using the CHRO Magar Orientation and biochemical test in our study

Strains of bacteria	Biochemical Test						
	Percentage %	Gram stain	Indole	Citrate	Urease	Motility	Colour on CHROM agar
1-E. Coli	64.28	G-ve	+	—	—	+	Dark violet
2-*K. pneumonia*	19.28	G-ve	—	+	+	—	Metallic blue
3-*P. mirabilis*	9.28	G-ve	—	—	+	+	Brown
4-*P. aeruginosa*	3.57	G-ve	—	+	+	+	No work

Obtained results in our study have shown that CHROM agar ™ Orientation media is applicable in the differentiation and primary recognized for bacteria from urine based on and morphology of colonies Table 1. As reported by other studies, CHRO Magar ™ Orientation was found to use as a simple and trusted method, resulting leading to saving cost, time and labor, addition to these necessary tests such biochemical are straightforward and trusted to distinguish between isolates from a urine samples[21,22,23,24]as shown in (Fig.1).

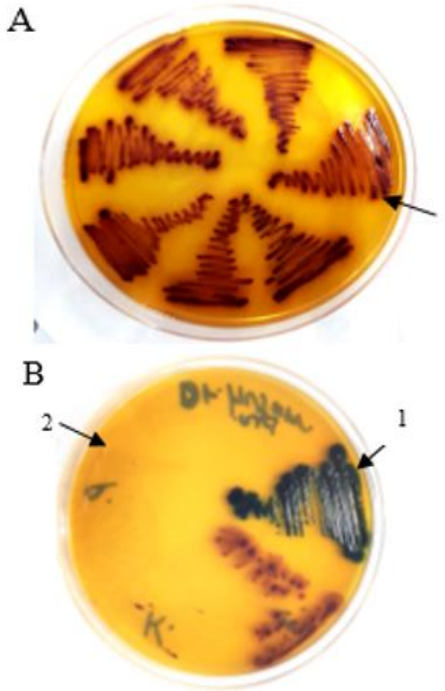

Figure 1. A: *E. coli* (Dark violet), B: 1-*K. pneumonia* (Metallic blue), 2- *P. mirabilis* (Brown).

Data show the resistance number and percentage of uropathogenic *E. coli* isolates to the antibacterial agents used in update study. We discovered that the antibiotic resistance result reveals max percentage resistance of these bacteria from clinical samples to 13 antibiotics. current study revealed that *E.coli* isolates had high level of resistance to Nalidixic acid (84.44%), and variable percentage of resistance to Gentamicin (71.11%), Ceftriaxone (78.88%), Cefotaxime (72.22%), Amikacin (68.88%), Ceftazidime (64.4%), Cefoxitin (22.22%), Ciprofloxacin (64.44%), and Aztreonam (70%), Nitrofurantoin (31.11%), Trimethoprim-Sulfamethoxazole (71.11%), and Augmentin (58.88%), and only low level of resistance to Imipenem (6.66%) depended on CLSI 2015 according to a diameter of inhibition zone mm. while Sensitive of isolates to antibiotics as follow Amikacin (12.22%), and variable percentage of sensitive to Gentamicin (28.88%), Ceftriaxone (21.11%), Cefotaxime (27.77%), Nalidixic acid (115.55%), Ceftazidime (35.55%), Cefoxitin (77.77%), Ciprofloxacin (35.55%), and Aztreonam (30%), Nitrofurantoin (68.88%), Trimethoprim-Sulfamethoxazole (28.88%), and Augmentin (41.11%), and only high level of to sensitive Imipenem (93.33%) (Fig. 2) Show percentage of all isolates.

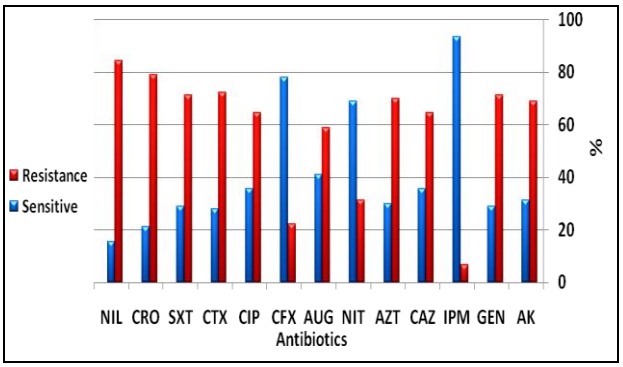

Figure 2. Antibiogram profile of UPEC isolates by disk diffusion method.

Results from Table.2 show that lower resistant percentage was found for Imipenem (6.66%), this result agrees with a previous study by [25]while disagreeing in another study when all isolates are sensitive to imipenem (100%) achieved by[26]. The imipenem, knowing as ground-breaking antimicrobial specialist's extreme diseases resulting from drug resistance of almost gram-negative bacteria. As per our observation, carbapenems stayed to be the strongest antibiotic against *Enterobacteriaceae* the event of carbapenemase has tossed out a test to antimicrobial treatment choices[27]. *E. coli* showed high resistance to Aminoglycosides included Amikacin, Gentamicin (68.8%, 71.1%) respectively that was agreed to study in Bangladesh [28]. Gentamicin and Amikacin are low cost, utilized parentally and fewer side effects reactions except for nephrotoxicity; however, this study uncovers a high opposition rate, separately to *E. coli*. While another study, the enzymatic reaction of resistance against Aminoglycosides to *E. coli* in Poland discovered that Gentamicin and Amikacin resistance rate was lower[29].

Table 2. Antibiotic Susceptibility of 90 *E. coli* isolates.

Antibiotics	NIL	CRO	SXT	CTX	CIP	CFX	AUG	NIT	AZT	CAZ	IPM	GEN	AK
R	76	71	64	65	58	20	53	28	63	58	6	64	62
N(%)	84.44	78.88	71.11	72.22	64.44	22.22	58.88	31.11	70	64.6	6.66	71.11	68.88
S	14	19	26	25	32	70	37	62	27	32	84	26	28
N(%)	15.55	21.11	28.88	27.77	35.55	77.77	41.11	68.88	30	35.55	93.33	28.88	31.11
Total	90	90	90	90	90	90	90	90	90	90	90	90	90
N (%)	100	100	100	100	100	100	100	100	100	100	100	100	100

R=Resistance, S=Sensitive, N%=Number of percentage

The high rate of resistance to Aztreonam (70%) that was near consequence of study accomplished in Iran[30]. However, it is conflicted with another study directed in Brazil with the opposition rate (82.8%) higher than our outcome[31]. In the present research, the result of resistance to Ceftazidime was (64.4%). This outcome is in concurrence with the investigation of [32.] Who revealed that the rate resistance of between *E. coli* was toward Ceftazidime (64.3%). On the other hand, in study [33]high rate of resistance was recognized against Ceftazidime (100%). Also, an agreement to our result in resistance to Nitrofurantoin with study archived by[34].While disagreement in other research accomplished in Jerusalem was the rate of resistance to Nitrofurantoin (12.7%)[35], a few supportive conclude can be drawn from our outcomes concerning antimicrobial decisions in this gathering. 1- Exposure to a past antibacterial specialist is of significance for a 6month period just; in this manner of UTIs ought not to modify the rule-based methodology 2-Nitrofurantoin displayed low connection with future resistance for another antibiotic with almost illness people are suffering weakness for operator regardless of whether pretreated with different classes[36]. Augmentin resistance in our finding showed (58.88%) that has an agreement to study conducted in Kerman[37]. But a lower rate of resistance in another study in Tabriz approximately (36.6%)[38]. The explanation behind this resistance from generally utilized antimicrobials might be because of far-reaching and aimless use in our condition[39]. As showed in table2, resistance rates for Cefoxitin were (22.2%) compared with our result according to[40]. But, they were resistance rates (9.4%) in Duhok less than what was found in our study[41]. The reason behind that are enzymes of lactamase that showed in 20 *E. coli* isolates resistant to Cefoxitin in percentage (22.2%) in our study[42]. The result from Table 4 shows the rate of resistance to Ciprofloxacin (64.44%) that was very close to the paper accomplished in India[43] and in a different low rate of resistance (14.5%) to study achieved in Ethiopia[44]. The genetic elements, such as plasmids responsible for antibiotic resistance ciprofloxacin possibly relocate between microorganisms by process gene transfer[45]. Third-generation cephalosporins Cefotaxime resistance rate of the *E. coli* from UTI was (72.22%) close enough to result of the study in Mexico [46]. But different to result obtained for cefotaxime (78%) was higher than our result in presented study [47]. As well, the resistance rate to Ceftriaxone was (78.88%) similar to results of a study accomplished in Egypt [48]. While lower than our result, approximately (73%) by *E. coli* from urine isolate in Ethiopia [49]. The emergence of a max level of reluctance to Cephalosporins for these bacteria from the urine is a product of extended beta-lactamase that leading to useless therapy of this disease, adding to that ability of *E.coli* to develop resistance to this generation [50]**.** Results from table.2 show that resistant percentage was found for Trimethoprim-Sulfamethoxazole (71.1%). This result agrees with a previous study by [51]. *E. coli* isolates are resistant in the study achieved by [52.] It has (78.7 %) higher than our finding. The resistance rate was (84.4%) for Nalidixic acid that similar to the resistance rate in the study of Nigeria[53]. But, it has meager resistance rate (31.9 %) for Nalidixic acid when compared to our finding to study in France [54].

As shown in schedule 3, the result of Antibody levels with a mean (425.2 ±17.3) at 14 days from fortified. Then, the Antibody level reaches to the highest value of mean (685.3 ±49.8) at 28 days. The outcome of values appeared a significant increase of IgG level at p-value of least of (0.05 value) from 14 to 42 days, as compared against the control group as shown in Figure 3.

Schedule 3. Antibody IgG level in serum of mice by Elisa during 42 days

Sample (Days)	The immunized group with UPEC vaccine Mean ± SE	Control group Mean ± SE
0	193±11.1 A	188.9 ±10.2 A
14	425.2 ±17.3 A	201 ±11.2 B
28	685.3 ±49.8 A	190.1 ±12.1 B
42	558.6±2.93 A	189 ±10.1 B

Same letters in the same row = (no different significant), Differ letters in the same row = (different significantly).

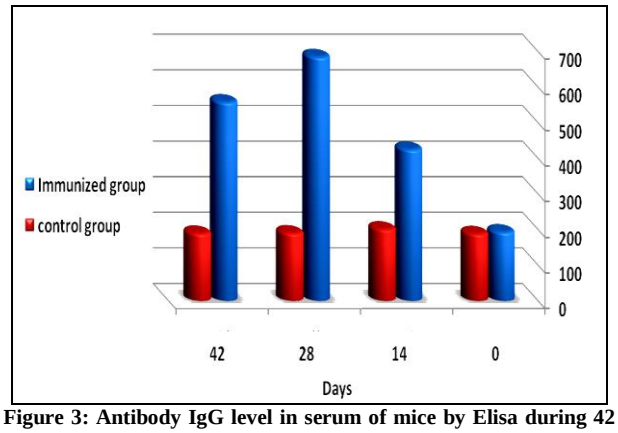

Figure 3: Antibody IgG level in serum of mice by Elisa during 42 days

In this case, fortified experiment animals against prepared vaccine leading to provoke of the immune system in the fortified group as comparable with non-fortified animals. That very close to result of the study which showed the investigation of the work of humoral immunity against the pathogen and the level of the antibody in addition to the contribution of the complex system improves the production of antibodies, which explains the high levels of the antibody and the suppressed effect against the pathogen[55]. This state whole cell bacteria killed by heat with antiserum can be immune therapy to mice that explained simple variation in levels of IgG.

While all outcome for this test shown an excess in the density of the pad of mice feet skin of the fortified group at 24 hrs (4.240 ± 0.240) when compared with the control group, and the highest value to means of the density appear at 48 hrs. (5.021±0.210) with p-value of least of (0.05 value) listed in Table 4 and Figure 4

Table 4. The density of the pad of mice feet skin of the fortified group in DTH test.

Sample hrs.	Fortified animal with prepared vaccine Mean ± SE	Control group Mean ± SE
Zero hrs.	2.00 ± 0.118A	2.06 ± 0.0597A
24 hrs.	4.240 ± 0.240A	2.1 ± 0.0653B
48 hrs.	5.021 ± 0.210A	2.11 ± 0.0615B
72 hrs.	2.311 ± 0.110A	2.07 ± 0.0614B

Same letters in the same row = (no different significant)
Differ letters in the same row = (different significantly)

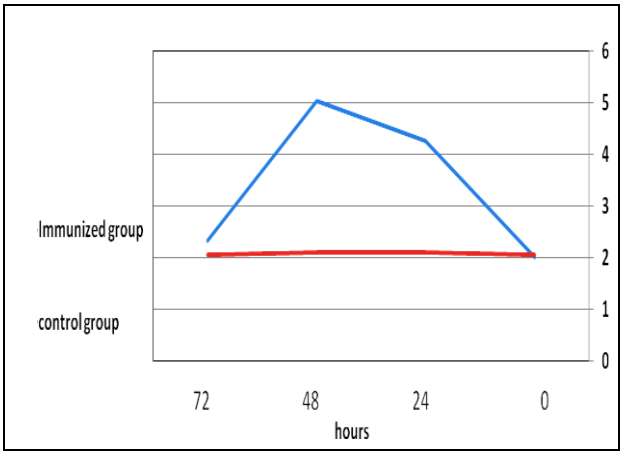

Figure 4. Skin thicknesses to fortified group with the control group

The prepared vaccine of whole cell bacteria includes several antigens that are capable of inducing cellular immunity. Delayed hypersensitivity test through at our methods is approved, and the result of the previous study when a prepared vaccine was used in the fortified mouse, a significant excess in the foot thickness was observed after 24 hours of injection in the right footpad and reached a high level of 48 hours compared to control group[56].

Delayed hypersensitivity tests rely on action T cell to distinguish pathogen with excreted interleukin-1 that leading to differentiation to T helper cell which excreted interleukin 2 as a chemo attractive signal to bring macrophage nearly the site of activated T cell which that excreted interferon that promotes the cytolytic action of aggregate macrophages leading to excess in the density of skin. In this search discover the prepared vaccine (whole cell bacteria) protected animal against infection by the intraperitoneal route. In a previous study, it showed incompatibility immunization used in several ways where the mice were fortified by the intraperitoneally but did not survive when using the intranasal method [57].

CONCLUSION:

This study investigates immunization explicitly in contradiction of multidrug resistance Uropathogenic *E. coli* isolate from urinary tract infection in pregnancy. After disease and immunization, ninety clinical isolates were recognized as UPEC obtained from patients from three hospitals in south Iraq and then investigated by biochemical test. Each isolate has been tested for susceptibility testing. One isolate has been selected for the immunization and vaccine model, the one remittent for most antibiotics. ELISA has adopted for assessments of immunoglobulin (IgG) level to serum mouse at 14, 28 and 42 days of immunization. Results have shown that isolate showed resistance to all antibiotics. There are significant differences when using UPEC in the level of IgG reaches the peak as compared with control for assessment humoral immune response. High significant differences thickness of the footpad skin after the complete day of the immunized mice, so that vaccine with this isolate has a potential for development as a candidate vaccine for protection against infection

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CONFLICT OF INTEREST:

No conflict of interest in this study.

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