Protective multi-epitope candidate vaccine for urinary tract infection

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**A B S T R A C T**

Urinary tract infections (UTIs) are induced by exogenous organisms including extraintestinal pathogenic such as Escherichia coli (ExPEC), Proteus mirabilis and Klebsiella pneumonia, which are closely related. These organisms can colonize in the urinary tract and cause UTIs. In this study, a cross-reactive multi-epitope vaccine was designed by two constructs to stimulate the immune system (CD8+ and CD4 + T cells) against ExPEC, Proteus mirabilis and Klebsiella pneumonia strains.

Uropathogenic Escherichia coli (UPEC), Proteus mirabilis and Klebsiella pneumoniae are the main bacterial cause of UTI. They were used for designing experimental candidate vaccine, and their immunogenicity and protective were assessed. In this study, conserved antigens from their bacterial genomes were considered, and informatics-based immunological vaccine with cross-protective T and B-cells epitopes was designed and evaluated. The vaccine candidate was used as a broad immune system inducer, and its cross-protective immunity and protective were confirmed in vivo experiments.

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1. Introduction

Urinary tract infection (UTI) is one of the most common infectious diseases [1]. As one of the common colonizer organisms in the human gastrointestinal tract, Escherichia coli is the major cause of UTIs being responsible for 90 % of the cases [2]. Microbial pathogens, such as extraintestinal pathogenic E. coli (ExPEC), along with other family members of Enterobacteriaceae, Proteus mirabilis and Klebsiella pneumoniae have been recognized as the common causes of infectious disease in the urinary tract in the community and healthcare settings [3]. Urinary tract infection (UTI) pathogens can result in a wide range of infections. ExPEC strains account for over 80 % of UTI cases; the intestinal pathogenic E. coli group including many E. coli pathotypes such as enterotoxigenic (ETEC), enteropathogenic, enterohemorrhagic (EHEC), enteroinvasive, adherent invasive, and diffusely adherent E. coli, may lead to infections in the human intestinal tract [1]. P. Mirabilis is responsible for 1–10 % of all recurrent UTIs; this pathogen is difficult to treat as 48 % of its strains are resistant to broad-spectrum of antibiotics [4–6]. Acquisition of antibiotics resistance can also derive from a wide range of K. pneumoniae strains [7]. As a consequence of antibiotic resistance, infections such as UTIs have become resistance to treatment giving rise to serious infections which may be life-threatening [7].

The majority of UTIs can be treated by antibiotics, but the drug resistant strains have raised a major challenge in treatment of UTI. This has increased the concerns on the deficient response to antibiotics in complicated UTIs, recurrent UTIs, bacteremia, and sepsis. Therefore, there is a need to develop efficient vaccines to prevent from UTIs. Development of conventional vaccines such as whole cell organisms (inactivated or live attenuated) are not recommended due to unnecessary antigenic load which may lead to the non-specific immune responses [8–10]. A subunit-based vaccine is a peptide-based or epitope-driven vaccine with different antigenic epitopes [8]. Peptide-based vaccines are highly specific and easy to design and formulate compared to whole cell or subunit vaccines [8–10]. With progress of bacterial whole genome sequencing and advances in bioinformatics, reverse vaccinology has been revolutionized [11,12]. Reverse vaccinology targets antigens based on their microbial genome sequence analysis. Vaccination inducing considerable protective immune response could not elicit with an antigen alone [13]. Therefore, the use of multi-subunit or multi-peptide approaches could be advantageous for delivery of high doses of antigens to develop protective immune responses [14]. Using reverse vaccinology in ExPEC and subtractive analysis of nonpathogenic antigen strains analysis, 9 antigens were found to be protective in a mouse challenge model [15]. In this way, development of a broadly protective E. coli vaccine could be possible [16]. The gene encoding showed the most of highly
conserved sequence and protective antigen in most of *E. coli* isolates [15].

In this study, bacteria causing UTI were considered as the most commonly bacterial pathogens: uropathogenic *Escherichia coli* (UPEC), *Proteus mirabilis* and *Klebsiella pneumoniae*. The bacterial virulence factors, such as adhesion, toxins, iron acquisition proteins, metabolic enzymes and structural components were considered for vaccine preparation. In this experiment, nine already-detected antigens in UPEC were searched by in silico studies in *Klebsiella pneumoniae*, and *Proteus mirabilis*. The common antigens were selected by various bioinformatics tools and the most common epitopes were selected. The multi-epitopes were synthesized and then expressed in *E. coli*. The immunogenicity and efficacy of the induced humoral and cellular responses (by the constructed fusion protein with adjuvant) was evaluated in mice models. The expressed proteins were then assessed for their in vivo protectivity in animal models. The aim was to design a candidate vaccine for stimulating the cellular immune system (CTL and T helper) against ExPEC, *K. pneumonia*, and *P. mirabilis* to concur a broad spectrum of UTI-inducing bacteria and recurring UTI infectious disease.

2. Materials and methods

2.1. Identification and selection of vaccine targets

Epitope prediction and selection was performed on nine candidate proteins [15]. The verified immunogenic epitopes were designed by in silico tools using available data from reference sequences [15]. In this experiment, conserved domains for each candidate were obtained from NCBI databases [17]. Epitope prediction was carried out using the IEDB (immune epitope database and analysis resource) server. IEDB is a free resource providing experimental data on characterization of antibody and T cell epitopes in humans, and non-human primates, as well as other animal species. Epitopes involved in infectious disease, allergy, autoimmunity and transplant were included. IEDB tools also assisted in prediction and analysis of B cell and T cell epitopes [18].

MHC class I and II prediction were conducted and the length of amino acid epitopes was selected [18]. Efficient epitopes were determined for each nine candidates. Then the selected epitopes were examined by Blast and total alignment in the whole genome of *K. pneumonia* and *P. mirabilis* strains. Common epitopes in *E. coli*, *K. pneumonia* and *P. mirabilis* strains were determined.

2.2. T helper and CTL construct

The significant pattern and frequency of each epitope were assessed by evaluating the immunization ability of the designed vaccines. In T helper (Th) and Cytotoxic T Lymphocytes (CTL) construct, the fusion of epitopes was assembled by Epitool Kit 2.0 server for the novel epitope-based candidate vaccine [19]. In the CTL construct, cholera toxin B subunit was selected as the adjuvant; while domains of flagellin antigen and (universal T helper pan DR epitopes) PADRE sequence served as the carrier adjuvants in T helper construct [20]. Adjuvants enhanced phagocytosis, up-regulation of MHC-I, survival of T cells and memory responses. Effect of linkers on stability, proper folding, structural flexibility and protein functional dynamics was also assessed. Alpha helix-forming linkers with the sequence of EAAK amino acids linker was chosen as the adjuvant carrier which was connected to both constructs. EAAK linker can increase the stability and folding of the constructs. Sequences of GPGP linker were selected in the T helper construct. Physicochemical characteristics of both CTL and T helper constructs were checked by ExPASy server (http://web.expasy.org/protparam/).

The constructs were designed with particular fusion of epitopes with proper adjuvant linkers, and carriers. Physicochemical characteristics, post-translational modifications (PTMs) solubility, allergenicity and antigenicity as well as reverse translation, codon optimization and second and tertiary structure prediction were also assessed for the two designed candidate vaccines (http://www.cbs.dtu.dk/services/, http://www.biocuckoo.org/, and http://web.expasy.org/) [21,22]. Reverse translation and codon optimization were evaluated by mEMBOSS 6.0.1 (http://www.ebi.ac.uk/Tools/st/emboss_backtranseq/) program; while second and tertiary structure prediction was carried out using I-TASSER server (Software Swiss-PdbViewer version 4.1.0).

The GenBank accession numbers were obtained for the newly designed T helper and CTL constructs (MG242033 and MG242034).

2.3. Synthesis of constructs

The designed multi-epitope synthetic genes were synthesized with HindIII restriction enzyme sites (Gene Fanavaran). Amplification of the genes was performed by designed forward and reverse primers with Polymerase Chain Reaction (PCR). PCR reactions were performed using Eppendorf thermo-cycler; PCRs were carried out in 50 μl volume containing 3 μl of DNA template, 2 μl of forward primer, 2 μl of reverse primer, 25 μl master mixture and 18 μl DDW (double-distilled water). The PCR condition for amplification of the synthetic gene included initial denaturation for 5 min at 95 °C, followed by 20 cycles involving 1 min at 95 °C, 1 min at 60 °C and a final extension at 72 °C for 1 min. The presence of the amplified products was evaluated by electrophoresis on 1% agarose gel. Plasmid vector, pET28a (Novagen) was digested with the restriction enzymes (Fermentas) and ligated at 22 °C overnight by T4 DNA ligase (Fermentas). The result was assessed by 1% agarose gel electrophoresis. Expression of recombinant proteins containing 6xHis tags in C-terminal was applied as mentioned in previously described protocols [21].

2.4. Bacterial strains

Antigens were selected based on different criteria from ExPEC (IHE3034, 536, and CFT073) and *P. mirabilis* HI4320 strain. TOP10 strain was used for the plasmid *Escherichia coli* transformation according to the manufacturer’s instructions [21]. Bacterial clones containing the recombinant plasmid were cultured. The plasmids were isolated and the presence of the desired fragment was examined by digestion assay according to the manufacturer’s protocol [21]. The Mac Conkey and agarose plates containing kanamycin (100 μg/μl) were used for selection of transformed colonies. The white transformed colonies were selected following an overnight culturing. They were then checked by plasmid extraction and PCR. The cloned fragments were sequenced as the selected recombinant plasmids (MWG DNA sequencing service, Biotech AG). Competent *E. coli* Top10 cells were transformed with the ligation mixture. Recombinant colonies were evaluated in 1% agarose by electrophoresis after digestion with the restriction enzymes (Fermentas).

2.5. PCR amplification

DNA of genomic constructs was transfected to the competent bacteria (Top10). It was then purified from bacteria cultured overnight (at 37 °C in humified 5% CO2) in LB (Difco). DNA concentration was calculated by optical density determination at 260 nm. Primers were designed in conserved DNA region and the genes were amplified using external primers. For the amplification, 100 ng of DNA was used as template. The amplification enzyme was Vent® DNA polymerase (Invitrogen). PCR conditions were as
performed on a SDS-PAGE gel and blotting was conducted into a nitrocellulose membrane. The membrane was blocked and the conjugated His-specific antibody was added (1:1000 dilution) for development. The expressed proteins were purified by His-tag affinity chromatography on Ni-NTA column (Qiagen) with a denaturation system applying triton X-114 to remove the lipopolysaccharide (LPS) from the recombinant proteins. The LPS level of the purified proteins was measured using the chromogenic Limulus Amebocyte Lysate test (LAL), according to the manufacturer’s protocol (Lonza). Then, the purified proteins were dialyzed against different concentrations of urea and the final concentrations were measured using Bradford assay with concentrated Bradford solution (BioRad).

2.7. Mice immunization and challenge studies

Female BALB/c mice of 6–8 weeks old (10 females per group, at least three groups per antigen) were purchased from Pasteur Institute of Iran. The animal tests were performed according to the European community council directive of 24 November 1986 (86/609/EEC). The mice were divided into 5 groups which underwent the injections (20 μg of recombinant protein in 150 μL of saline solution) three times with 2-week intervals (on days 1, 14, and 28). Negative control mice were immunized with saline solution. Immunized animals were challenged at day 49 with a lethal dose of homologous strains. Heparinized-blood samples were collected from the survived mice 20 h after challenge to determine their bacteremia levels; the mortality was monitored 4 days after challenge. The vaccinated mice were consequently used for cytokine and challenge experiments.

Antigen-specific serum and urine responses including total IgG, IgA and IgG isotypes (IgG1 and IgG2a) were determined by standard enzyme-linked immunosorbent assay (ELISA). In a typical procedure, the ELISA 96-well plates (Greiner, Germany) were coated with purified constructs (Th, CTL, and mixed constructs) (10 μg/mL in PBS) and incubated overnight. The plates were blocked with 3% bovine serum albumin (BSA) and incubated with two-fold serial dilutions of immune serum (1:50–1:6400) and mucosal samples (undiluted, 1:5 and 1:10) in 1% BSA. Afterwards, HRP-conjugated goat anti-mouse IgG, IgG1, IgG2a and IgA (Zymed) were used as secondary antibodies. The plates were incubated with the TMB substrate to visualize the antibody reactivity at 450 nm using an ELISA reader.

![Fig. 1](image-url)

**Fig. 1.** a) T helper (Th) construct with flagellin (FljB) and PADRE sequence as carrier. FljB: phase 2 flagellin, strain: Salmonella enterica subsp. Enteric aserovar Typhimurium str. LT2, TLRS agonist, gi|15676083|ref|NP_461698.1| PADRE: Universal T helper pan DR Epitope

Linkers: EAAAQ, GPGP, AAA

b) CTL construct with cholera toxin B subunit carrier. Cholera toxin B subunit, strain: Vibrio cholerae O1 biovar El Tor str. N16961, gi|15641467|ref|NP_231099.1|

HHHA: heparin binding hemagglutinin, strain: Mycobacterium tuberculosis H37Rv, gi|15607616|ref|NP_214093.1|, TL4R agonist

| Table 1 |
| --- |
| Sequence of MHC class I and MHC class II inducer epitopes were selected based on the lowest score by IEED, BioEdit, Xyte and Doolittle programs. |
| MHC class I antigen 1–9 |
| Antigen no. | Oligonucleotide sequence | Epitope Score |
| 1. | FIPPGHQFG | 9.3 |
| 2. | NICAYQFL | 4.5 |
| 3. | YMYGRGLGV | 0.4 |
| 4. | WMAQVNTFI | 0.8 |
| 5. | SVNPVLSDTIVNEI | 1.28 |
| 6. | SGCNQAGV | 1 |
| 7. | NLDFKTYYT | 0.4 |
| 8. | ILSDGTNTV | 0.5 |
| 9. | YLSGYGHHI | 0.5 |

| MHC II Antigens 1–9 |
| Antigen no. | Oligonucleotide sequence | Epitope Score |
| 1. | PDCLGRLSTIVSVL | 0.98 |
| 2. | DQYRSIBNITITWL | 0.70 |
| 3. | CNQLGMYRSQGLQVE | 2.05 |
| 4. | RNFIITGMATAKANQ | 1.25 |
| 5. | KQLKTILSVDNYYW | 1.04 |
| 6. | EARWFLSTTRWQND | 1.18 |
| 7. | QTTPQISSISIGD | 0.25 |
| 8. | IHLQDCLSDTNTV | 0.54 |
| 9. | NDUMYKAEGNLSI | 0.60 |

follows: 35 cycles of denaturation at 98 °C for 10 s, annealing at 55 °C for 20 s and elongation at 72 °C for 3 min. PCR products were then purified according to the manufacturer’s instructions [21].

2.6. Cloning and protein expression

PCR amplification was conducted from the genomic DNA templates cloned in pET28a vector (Novagen); they were then transformed in BL21 (DE3) competent cells for protein expression. Two multi-gene synthetic candidates were cloned and expressed as His-tagged fusion proteins and the proteins purified as previously described [21]. Synthesis of multi-epitope constructs was induced by adding different concentrations of isopropyl-beta-thio galactopyranoside (IPTG). Then, the protein expression was assessed by 15 % sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and confirmed by western blotting using monoclonal HRP-conjugated His-specific-antibody (Cyto Matin Gene). Briefly, recombinant proteins separation was performed on a SDS-PAGE gel and blotting was conducted into a nitrocellulose membrane. The membrane was blocked and the conjugated His-specific antibody was added (1:1000 dilution) for development. The expressed proteins were purified by His-tag affinity chromatography on Ni-NTA column (Qiagen) with a denaturation system applying triton X-114 to remove the lipopolysaccharide (LPS) from the recombinant proteins. The LPS level of the purified proteins was measured using the chromogenic Limulus Amebocyte Lysate test (LAL), according to the manufacturer’s protocol (Lonza). Then, the purified proteins were dialyzed against different concentrations of urea and the final concentrations were measured using Bradford assay with concentrated Bradford solution (BioRad).

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bodies for splenocytes. Analysis of variance (ANOVA), Student t-test, and Tukey HSD tests. Values below 0.05 were considered as significant. The virulence E. coli antigens were selected based on protective antigens studied in the mouse model [15]. Protective antigens including hypothetical proteins, c0975 (gi|26246865), c1275 (gi|26247149), and c5321 (gi|26251128) from Escherichia coli CT073, bacterial Ig-like domain (group 1) ECOK1_0290 (gi|386598028), general secretion pathway protein K ECOK1_3374 (gi|386600983), putative lipoprotein ECOK1_3385 (gi|386600994), TonB-dependent siderophore receptor ECOK1_3457 (gi|386601066), fimbrial protein ECOK1_3473 (gi|386601082) from Escherichia coli IHE3034 and hemolysin A (gi|110643969) from Escherichia coli 536 were considered. Amino acids sequences of the antigens were compared between P. mirabilis and K. pneumonia strains; the most MHC-I and MHC-II inducers were selected based on the lowest score from IEBD, BioEdit, Kyte, and Doolittle programs (Table 1). T helper (Th) and CTL constructs were designed as T helper with flagellin and PADRE sequence linker as a carrier adjuvant and CTL construct with cholera toxin B subunit carrier adjuvant (Fig. 1) [22–26].
3.2. Expression of candidate vaccine

Multi-epitope Th and CTL constructs were cloned into the pET28a vector. The constructs were expressed and the expression of constructed proteins (Th and CTL) was confirmed by western blotting (Fig. 2a and b). The constructs alone were successfully expressed in *E. coli* BL21/plysS and purified using a nickel chromatography column.

3.3. Antibody production

After immunization, the induced responses were assessed by different dilutions of IgG; total serum (1:50–1:6000) was obtained 2 weeks after the last injection. As shown in Fig. 3a, serum dilutions differentiated between the IgG levels in mice groups (Th and CTL). It was also observed that the IgG response by Th vaccine candidate was higher than CTL construct (\(p < 0.001\)). In addition, total IgG responses with Mixed Th and CTL constructs was significant comparing with controls (Th or CTL coated plates) (\(p < 0.01\)) and even the plate was coated with Th though CTL construct as shown in Fig. 3a.

A significant increase can be detected in IgG1 and IgG2 responses of Th (\(p < 0.0001\)) and CTL groups (\(p < 0.001\)) as compared with controls (Th or CTL coated plates) (Fig. 3b). However, a significant difference was observed between the IgG1 and IgG2 responses of the mixed Th (Th coated plate) and mixed CTL (CTL coated plate) groups (\(p < 0.001\)).

IgA antibody was measured in serum samples 2 weeks after the third vaccination. As shown in Fig. 4, in all vaccine combinations, significant responses were induced in Th group (\(p < 0.015\)) and mixed Th (\(p < 0.001\)) as compared to the control group. The induced mucosal immune responses were measured in the urine samples after the last immunization.

Considering the role of mucosal responses in the UTIs prevention, total IgG, IgG1, IgG2 and IgA antibody responses were significant in all groups.

3.4. Cytokines immune response

IFN-\(\gamma\), IL-17 and IL-4 were assessed in all groups. High levels of IL-4, IL-17 and IFN\(\gamma\) were produced in all the immunized groups compared to the controls (Fig. 5). Mice vaccinated with constructs showed cytokines production. While no significant difference was observed in production of IL-4 and IL-17 between the groups, there was a significant release of IFN-\(\gamma\) in the mixed CTL group (\(p < 0.009\)) compared to CTL group (\(p < 0.039\)).

3.5. Immune protection and challenge in bladder and kidney

The efficacy of the induced immune responses in bladder and kidney infection was evaluated by the mice groups challenged by standard strains of *UPEC*, *P. mirabilis*, and *K. pneumonia* infection. As Fig. 6 suggests, the bacterial load in the bladders and kidneys of all groups showed approximately 10–100 folds decreased compared to the controls (\(p < 0.05\)).

4. Discussion

As a common cause of UTI, *E. coli* is responsible for more than 85% of UTIs. Their incidence rank differs country by country; however roughly 130–175 million patients are diagnosed with UTI worldwide [27]. Approximately, half of women experience UTI, 25% of them will suffer recurrent UTI [27]. UTIs can also lead to pyelonephritis, bacteremia, and sepsis. Antibiotic is generally the first and effective step in treatment of infection. However, increase in antibiotic resistance has resulted in recurring infections and
alteration of normal gut flora. For more than a century, the purpose of vaccine usage against UTI was basically therapeutic rather than prophylactic. Different types of classical UTI vaccines such as attenuated, inactivated, subunit, toxoid and conjugate have been used so far. However, a limited number of modern vaccines have been tested in humans, and only one is currently commercially available [28]. To the best of our knowledge, there are commercial vaccines such as Uro-Vaxom (OM Pharma, consisting of 18 uropathogenic strains), Solco-Urovac (a mixture of heat-killed uropathogenic E. coli; comprising 6 strains), Urvakol and Urostim (which contain E. coli, P. mirabilis and E. faecalis strains but Urvakol also includes a Pseudomonas aeruginosa strain, whereas Urostim contains K. pneumoniae). Both Uro-Vaxom and Solco-Urovac vaccines were tested on humans.

Basically, there are two types of UTI vaccines; “whole cell” and “specific-antigen” vaccines. The former includes whole bacteria (either live attenuated or inactivated) or bacterial lysates while the latter encompasses one or more antigens (subunit, toxoid, or conjugate vaccines).

E. coli-induced UTI infections are an important part of vaccine controlling infection. E. coli and K. pneumoniae are the predominant bacteria involved in recurrent UTI. One of the most effective approaches to prevent UTIs is to design a potent immunogenic vaccine which could be well tolerated and reduce the frequency of UTI recurrence.

Isolated antigens typically do not elicit powerful or long-lasting immune responses; thus their inflammatory response must be increased by enhancing immunogenicity [29]. Moreover, the anticipated UPEC virulence factors were found in about 50 % or fewer of all isolates which would make a multi-subunit vaccine as the most powerful one. Bioinformatics have been also applied to predict B- and T-cell epitopes of antigens to increase the protective immunity, due to the possible role of B-cell (humoral response) and T-cell (cellular response) in protection against UTIs [30].

In our study, the selected common antigen epitopes in virulence pathogenic E. coli were compared with the highly conserved epitopes of P. mirabilis and K. pneumoniae strains. Linkers and carrier adjuvants were selected for multi-domain epitope antigens and their accurate function [31–35]. Adjuvant linkers were used to enhance the immune response and shift the responses to the Th1 direction as an internal adjuvant [36]. Induction ability of both humoral (Th2) and cellular (Th1) responses were assessed against Th and CTL constructs first computationally and then experimentally. In this study, the multi-epitope vaccine candidates were designed for the most UTI infection coverage. Furthermore, carrier linker adjuvants were also employed as an internal adjuvant for the first time.

The candidate vaccine in this study decreased the renal bacteria load and disease severity in mice after their challenging with a UPEC, P. mirabilis, K. pneumoniae standard strains. Vaccination managed to protect both the bladder and the kidneys after challenge.

In this experiment, it was found that the designed (Th and CTL) constructs immunization induced IgG1 (Th2) and IgG2a (Th1) responses without using common adjuvants. High levels of IgG1 and IgG2a (the long-lasting antibodies) induction was also observed. Moreover, as IgA is the most effective means of inducing mucosal immunity, it is known to be protective against invasive infection of the gut. In this experiment, antigen-specific IgA responses and protection were also assessed in the UTI mice model. In the present study, the multi-epitope vaccine constructs induced IFN-γ and IL-4 production which are indicators of Th1 and Th2 responses, respectively. A mixture of Th1 and Th2 responses could be also due to the recruitment of the antigen presenting cells (APCs), T CD4+ cells and CD8+ which resulted in high levels of Th1 and Th2 cytokines production. A significant protection was also observed in bladder and kidneys of the models during their challenging with vaccine constructs. The highest protection was detected against UPEC, P. mirabilis, K. pneumoniae strains in the bladder and kidney. Such significant protection could be attributed to the high mucosal and systemic immune responses. The protection efficacy was lower in kidneys of vaccinated mice compared to their bladder which may be due to the decrease in IL-17 levels or other immunological mechanisms.

Most of tested UTI vaccines use UPEC strains as uropathogenic E. coli is responsible for approximately 85 % of uncomplicated UTIs [37]. Development of new and effective therapies is of high priority in UTI research, as such therapies could positively affect the quality of life of millions of individuals and decrease the overall use of antibiotics. The significant contribution of this study is that the new candidate vaccine will likely eliminate the recurrent UTI in patients. In the absence of vaccination, UTI infections pathogens...
were able to predominate in the urogenital niche of the subjects, vaccination however overcame this issue.

Moreover, protection was enhanced by substitution of internal adjuvants (i.e. addition of cholera toxin adjuvant).

Further randomized controlled trials are necessary to determine the true clinical benefit of candidate vaccine. Recurrent UTI treatment is not adequately addressed by antibiotics; therefore, new vaccines with choice of internal adjuvant can resolve the limitations of vaccine efficacy and success.

5. Conclusions

The present study reports development of a novel multi-epitope candidate vaccine based on the common virulence epitope
antigens of *E. coli*. The efficacy of the mentioned vaccine was significantly shown. Furthermore, the findings of the present study suggest that internal carrier adjuvants could be also used as an alternative for traditional adjuvants especially for vaccine against UTI pathogens. Further clinical trials are required to present the candidate vaccine as a potential powerful candidate against UTI infections.

UTIs are generally induced by exogenous organisms including extraintestinal pathogenic *Escherichia coli* (ExPEC), *Proteus mirabilis* and *Klebsiella pneumonia*, which are closely related strains. These organisms are able to colonize the urinary tract and cause UTIs. In this study, a cross-reactive multi-epitope vaccine was designed by two constructs to stimulate the immune system (CD8+ and CD4+ T cells) against ExPEC, *Proteus mirabilis* and *Klebsiella pneumonia* strains.

We have developed a potential vaccine through in silico strategy, using anti-virulent compounds to make a multi-epitope vaccine. The designed constructs managed to induce significant immunity and protection against UTI infection. The candidate vaccine reported here is able to reduce bacterial burdens in a murine model of UTI. The candidate vaccine could provide a potential multi-epitope vaccine to prevent from bacterial pathogenesis and resistant infections.

**Contribution to authorship**

Study design: MO, SB; recruitment and enrolment of participants: MO; acquisition of data: MO, MRK; bioinformatics: MJ; biostatistical analyses: MRAK; study supervision: SB; drafting of the manuscript: JK, ZH.

**Declaration of Competing Interest**

The authors reported no declarations of interest.

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**Appendix A. Supplementary data**

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.btre.2020.e00564.

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