There is therefore a need in the art for new vaccines/ therapies/ therapeutics capable of specifically addressing *C. difficile* infection (CDI). This need is addressed by the present invention, which solves one or more of the above-mentioned problems.

Summary

In one aspect, there is provided a polypeptide, consisting of or comprising an amino acid sequence having at least 80% sequence identity with an amino acid sequence consisting of residues 1400-1800 of a C. *difficile* Toxin A sequence or a C. *difficile* Toxin B sequence; with the proviso that the polypeptides does not include one or more of the Repeat Unites (RU) located between amino acid residues 1851-2710 of C. *difficile* Toxin A and/or residues 1853-2366 of a C. *difficile* Toxin B.

In another aspect, there is provided a polypeptide according to the present disclosure, for use an antigen in a method of generating an antibody that binds to a C. *difficile* Toxin A and/or to a C. *difficile* Toxin B; preferably wherein said antibody comprises whole IgG, and Fab antibody or an $F(ab')_2$ antibody.

In another aspect, there is provided an *in vitro* method for isolating antibodies that bind to C. *difficile* Toxin A and/or to C. *difficile* Toxin B, said method comprising a) immobilising on a surface (for example on a matrix within a column) one or more C. *difficile* polypeptide(s) according to the present disclosure; b) contacting the immobilised polypeptide(s) with a solution containing antibodies that bind to . *difficile* Toxin A and/or Toxin B; c) allowing said antibodies to bind to said polypeptide(s), thereby forming a bound complex of antibody and polypeptide(s); d) washing away any unbound antibody or protein; and e) eluting the bound antibodies from the surface, thereby providing affinity-purified antibodies.

In another aspect, there is provided an antibody that binds to a C. *difficile* Toxin A and/ or to a *C. difficile* Toxin B, wherein said antibody is obtainable by a method according to the present disclosure, and wherein said antibody does not substantially bind to the effector domain of a *C. difficile* Toxin A or Toxin B and/ or does not substantially bind to a RU of a *C. difficile* Toxin A or Toxin B; preferably wherein said antibody comprises whole IgG, an Fab antibody or an $F(ab')_2$ antibody.

In another aspect, there is provided a polypeptide according to the present disclosure and/ or an antibody according to the present disclosure, for use in the prevention, treatment or suppression of CDI in a mammal (e.g. a human).

In another aspect, there is provided use of a polypeptide according to the present disclosure and/ or an antibody according to the present disclosure in the manufacture of a medicament for prevention, treating or suppressing CDI in a mammal (e.g. a human).

In another aspect, there is provided a polypeptide for use according to the present disclosure, wherein said use includes administration of said polypeptide as part of a combination therapy with one or more additional antigen selected from a *C. difficile* non-Toxin antigen, an inactivated or attenuated whole cell *C. difficile* bacterium, and/ or a *C. difficile* cell extract; and

a. optionally one or more antigen selected from 1) an antigen from a bacterium that causes nosocomial infection, and 2) an inactivated or attenuated whole cell bacterium that causes nosocomial infection; and

b. optionally one or more antibiotic, such as an antibiotic effective against a bacterium that causes nosocomial infection.

In another aspect, there is provided use of an antibody according to the present disclosure in an *in vitro* immunoassay method for confirming the presence or absence of a *C. difficile* infection in a patient sample, wherein the presence of a *C. difficile* infection is confirmed by detecting the binding of said antibody to a *C. difficile* Toxin A and/ or Toxin B present in said sample, and wherein failure to detect the binding of said antibody to a *C. difficile* Toxin A and/ or Toxin B present in said sample confirms the absence of a *C. difficile* infection.

In another aspect, there is provided use of a polypeptide according to the present disclosure in an *in vitro* immunoassay method for confirming the presence or absence of a *C. difficile* infection in a patient sample, wherein the presence of a *C. difficile* infection is confirmed by detecting the binding of said fusion protein to an antibody present in said sample, and wherein failure to detect the binding of said

fusion protein to an antibody present in said sample confirms the absence of a *C. difficile* infection.

In summary, the present ~~invention~~disclosure provides antigens that are able to induce a potent toxin-neutralising response against C. *difficile* Toxin A and/ or B. The ~~invention~~present disclosure also provides methods for preparing recombinant antigens, and the use thereof as immunogens to enable the large-scale preparation of therapeutic antibodies. Said antibodies are able to induce a potent toxin-neutralising response against C. *difficile* Toxin A and/ or B and therefore have prophylactic and/ or therapeutic applications.

Description

As mentioned above (see, for example, WO 00/61762), previous studies describe vaccine preparations based on the C-terminal, repeating units (RUs) of Toxin A and/ or Toxin B. Said RU fragments have a poor toxin-neutralising effect, and/ or are difficult to manufacture in large quantities.

In contrast, the present invention provides a C. *difficile* polypeptide antigen based on a Toxin A and/ or a Toxin B that does not contain or include one or more (e.g. all) of the repeating units (RUs) of Toxin A and/ or Toxin B. The polypeptide antigens of the invention consist of or comprise one or more domain from the central region of the toxins. Said antigens of the invention demonstrate good toxin-neutralising immune responses and/ or are readily manufactured in large quantities.

The present inventors have surprisingly identified that *C. difficile* antigen polypeptides which consist of or comprise one or more domain from the central region between the effector domain and the region of RUs (see Figure 1) provide a protective (toxin-neutralising) immune response that was greatly enhanced as compared to corresponding *C. difficile* toxin fragments comprising one or more of the RUs (see Tables 1 and 2). In one embodiment, the C. *difficile* antigen polypeptide comprises a Toxin B fragment.

Comparison of the data in Tables 1 and 2 confirms that the polypeptide antigens of the present invention elicit a considerably more potent toxin-neutralising immune

13. ~~A method for preventing, treating or suppressing CDI in a mammal (e.g. a human), said method comprising administering a therapeutically effective amount of~~ Use of a polypeptide according to any of Claims 1-7 and/ or an antibody according to Claim 10 or Claim 11 in the manufacture of a medicament for preventing, treating or suppressing CDI in a mammal (e.g. a human).

14. The ~~method~~ use according to Claim 13, wherein said polypeptide is to be administered as part of a combination therapy with one or more additional antigen selected from a *C. difficile* non-Toxin antigen, an inactivated or attenuated whole cell *C. difficile* bacterium, and/ or a *C. difficile* cell extract; and

a. optionally one or more antigen selected from 1) an antigen from a bacterium that causes nosocomial infection, and 2) an inactivated or attenuated whole cell bacterium that causes nosocomial infection; and

b. optionally one or more antibiotic, such as an antibiotic effective against a bacterium that causes nosocomial infection.

15. The ~~method~~ use according to Claim 14, wherein said antibody is to be administered as part of a combination therapy with one or more additional antibodies selected from an antibody that binds to a *C. difficile* non-Toxin antigen, and an antibody that binds to a whole cell *C. difficile* bacterium; and

a. optionally one or more an antibody selected from 1) an antibody that binds to antigen from a bacterium that causes nosocomial infection, and 2) an antibody that binds to a whole cell bacterium that causes nosocomial infection; and

b. optionally one or more antibiotic, such as an antibiotic effective against a bacterium that causes nosocomial infection.

16. A polypeptide for use according to Claim 12, wherein said use includes administration of said polypeptide as part of a combination therapy with one or more additional antigen selected from a *C. difficile* non-Toxin antigen, an inactivated or attenuated whole cell *C. difficile* bacterium, and/ or a *C. difficile* cell extract; and

a. optionally one or more antigen selected from 1) an antigen from a bacterium that causes nosocomial infection, and 2) an inactivated or attenuated whole cell bacterium that causes nosocomial infection; and

b. optionally one or more antibiotic, such as an antibiotic effective against a bacterium that causes nosocomial infection.