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 protective polypeptide antigen, 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 protective polypeptide antigen 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 and neutralises 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 protective polypeptide antigen 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 protective polypeptide antigen 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 protective polypeptide antigen 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 protective polypeptide antigen 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

CLAIMS

1. 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 polypeptide does not include one or more of the Repeat Units (RU) located between amino acid residues 1851-2710 of *C. difficile* Toxin A and/ or residues 1853-2366 of a *C. difficile* Toxin B.

2. The polypeptide according to Claim 1, wherein said polypeptide does not include the amino acid sequence provided by amino acid residues 1851-2710 of *C. difficile* Toxin A and/ or amino acid residues 1853-2366 of a *C. difficile* Toxin B.

3. The polypeptide according to Claim 1 or Claim 2, wherein the polypeptide consists of or comprises an amino acid sequence having at least 80% sequence identity with an amino acid sequence consisting of residues 1330-1850 or 770-1850 or 542-1850 of a *C. difficile* Toxin A sequence.

4. The polypeptide according to any preceding claim, wherein the polypeptide consists of or comprises an amino acid sequence having at least 80% sequence identity with an amino acid sequence consisting of residues 1145-1852 or 767-1852 or 543-1852 of a *C. difficile* Toxin B sequence.

5. The polypeptide according to any preceding claim, wherein said polypeptide further comprises a second amino sequence selected from one or more of a bacterial surface layer protein, NusA, thioredoxin, maltose-binding protein, a ubiquitin-like peptide, and a fragment of any of said second amino acid sequences.

6. The polypeptide according to Claim 5, wherein the second amino acid sequence comprises a *C. difficile* surface layer protein.

7. The polypeptide according to Claim 6, wherein the *C. difficile* surface layer protein consists of or comprises a CD 2767 peptide, for example a peptide consisting of or comprising an amino acid sequence that has at least 80% sequence identity with amino acid residues 27-401 of a CD 6767 peptide.

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8. A polypeptide according to any preceding claim, for use as 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, an Fab antibody or an $F(ab')_2$ antibody.

9. 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 any of Claims 1-7;

b) contacting the immobilised polypeptide(s) with a solution containing antibodies that bind to *C. 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.

10. 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 Claim 9, 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.

11. An antibody according to Claim 10, wherein said antibody binds to an epitope presented by residues 1350-1850 or 1130-1850 or 770-1850 or 542-1185 of a *C. difficile* Toxin A and/ or by residues 1350-1852 or 1145-1852 or 767-1852 or 543-1852 of a *C. difficile* Toxin B.

12. A polypeptide according to any of Claims 1-7 and/ or an antibody according to Claim 10 or Claim 11, for use in the prevention, treatment or suppression of CDI in a mammal (e.g. a human).

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13. 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 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 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.

17. An antibody for use according to Claim 12, wherein said use includes administration of said antibody 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.

18. Use of an antibody according to Claim 10 or Claim 11 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.

19. Use of a polypeptide according to any of Claims 1-7 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.

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CLAIMS

1. A protective polypeptide antigen, 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 polypeptide does not include one or more of the Repeat Units (RU) located between amino acid residues 1851-2710 of *C. difficile* Toxin A and/ or residues 1853-2366 of a *C. difficile* Toxin B.

2. The protective polypeptide antigen according to Claim 1, wherein said polypeptide does not include the amino acid sequence provided by amino acid residues 1851-2710 of *C. difficile* Toxin A and/ or amino acid residues 1853-2366 of a *C. difficile* Toxin B.

3. The protective polypeptide antigen according to Claim 1 or Claim 2, wherein the polypeptide consists of or comprises an amino acid sequence having at least 80% sequence identity with an amino acid sequence consisting of residues 1330-1850 or 770-1850 or 542-1850 of a *C. difficile* Toxin A sequence.

4. The protective polypeptide antigen according to any preceding claim, wherein the polypeptide consists of or comprises an amino acid sequence having at least 80% sequence identity with an amino acid sequence consisting of residues 1145-1852 or 767-1852 or 543-1852 of a *C. difficile* Toxin B sequence.

5. The protective polypeptide antigen according to any preceding claim, wherein said polypeptide further comprises a second amino sequence selected from one or more of a bacterial surface layer protein, NusA, thioredoxin, maltose-binding protein, a ubiquitin-like peptide, and a fragment of any of said second amino acid sequences.

6. The protective polypeptide antigen according to Claim 5, wherein the second amino acid sequence comprises a *C. difficile* surface layer protein.

7. The protective polypeptide antigen according to Claim 6, wherein the *C. difficile* surface layer protein consists of or comprises a CD 2767 peptide, for

example a peptide consisting of or comprising an amino acid sequence that has at least 80% sequence identity with amino acid residues 27-401 of a CD 2767 peptide.

8. A protective polypeptide antigen according to any preceding claim, for use as 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, an Fab antibody or an $F(ab')_2$ antibody.

9. 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 any of Claims 1-7;

b) contacting the immobilised polypeptide(s) with a solution containing antibodies that bind to *C. 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.

10. An antibody that binds to and neutralises a *C. difficile* Toxin A and/ or a *C. difficile* Toxin B, wherein said antibody is obtainable by a method according to Claim 9, 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.

11. An antibody according to Claim 10, wherein said antibody binds to an epitope presented by residues 1350-1850 or 1130-1850 or 770-1850 or 542-1185 of a *C. difficile* Toxin A and/ or by residues 1350-1852 or 1145-1852 or 767-1852 or 543-1852 of a *C. difficile* Toxin B.

12. A protective polypeptide antigen according to any of Claims 1-7 and/ or an antibody according to Claim 10 or Claim 11, for use in the prevention, treatment or suppression of CDI in a mammal (e.g. a human).

13. Use of a protective polypeptide antigen 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 use according to Claim 13, wherein said protective polypeptide antigen 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 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 protective polypeptide antigen 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.

17. An antibody for use according to Claim 12, wherein said use includes administration of said antibody 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.

18. Use of an antibody according to Claim 10 or Claim 11 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.

19. Use of a protective polypeptide antigen according to any of Claims 1-7 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.