Treatment of Bacterial Infections

The present invention is concerned with compounds, medicaments and treatments for *Clostridium difficile* infection, together with novel isolated antibodies and their use in same. The present invention is also concerned with the prophylaxis and treatment of *E.faecium* and *E.faecalis* infection and provides medicaments and treatments for same.

C. difficile is a gram-positive anaerobic bacterium, and is deemed a significant human pathogen causing a spectrum of diseases ranging from mild diarrhoea to fulminant pseudomembranous colitis (PMC) - collectively referred to as *C. difficile* antibiotic-associated diarrhoea (CDAD). CDAD is a common, iatrogenic, nosocomial disease associated with substantial morbidity and mortality, especially in the elderly. Two factors have been assigned main roles in the pathogenesis of CDAD - the suppression of the resident intestinal flora by the administration of antibiotics, and the production by the bacterium of two high molecular weight toxins, exotoxin A and exotoxin B.

The bacterium is endemic in hospitals, and studies have shown that approximately one third of patients receiving antibiotic treatment in acute-care medical wards were colonised by *C. difficile* while in hospital (Kyne, L., *et al.*, 2002, Clin. Infect. Dis. 34(3), pp346-53, PMID: 11774082). Of these patients, over half went on to develop CDAD while the remainder were symptomless carriers. CDAD is a major factor in extension of patient hospital stay times, and estimates suggest that the cost of this disease in the US exceeds $1.1 billion per year (Kyne, L., *et al.*, Supra). Patients suffering from CDAD respond well to a treatment which includes a discontinuation of the inciting antibiotic and treatment with either of the antibiotics metronidazole and vancomycin. The response rate to initial antibiotic therapy can be high (up to 95%), but up to 20% of patients can relapse within one or two weeks of a first treatment, with the risk of relapse compounding with each

relapse episode. Relapse can typically be treated with antibiotics, indicating that it is due to infection with different *C.difficile* strains. Additionally, there is evidence that *C. difficile* is becoming resistant to metronidazole and partially resistant to vancomycin, demonstrating the need for new alternatives in the treatment of CDAD.

Exotoxins A and B which are produced by pathogenic strains of the bacterium are cytotoxic, enterotoxic and proinflammatory, and are considered to be the main virulence factors of this non-invasive microorganism. However, not all infections with toxigenic strains result in disease, prompting the search for additional virulence factors. Bacterial surface expressed antigens represent candidate virulence factors, and are also considered important since such proteins likely mediate the essential functions such as adhesion to the epithelial layer of the gut in the first step of colonization or interaction with mediators of local immunity. In common with many other bacteria, *C. difficile* expresses a crystalline or paracrystalline surface layer (S-layer) on the outer cell surface. Such S-layers comprise proteins or glycoproteins forming a regularly arranged lattice on the external surface of the bacterium, and have previously been shown to be essential for the virulence of pathogens such as *Aeromanas salmonicida* and *Campylobacter fetus*. In contrast to most bacteria which comprise one S-layer, *C. difficile* is known to comprise two superimposed paracrystalline S-layers, each composed of a glycoprotein subunit which varies slightly in apparent molecular weight among different *C. difficile* strains. Most strains of *C. difficile* express two major S-layer proteins (SLPs), one of 32-38 kDa (low-MW SLP) and a second of 42-48 kDa (high-MW SLP). The low-MW SLP appears to be immunodominant and is the antigen most commonly recognised by patients suffering from CDAD, and is the only antigen recognised in EDTA extracts of bacteria by antisera raised in rabbits against whole *C. difficile* cells (Calabi, E. *et al.*, 2001, Mol. Microbiol., 40(5) p1187-99, PMID: 11401722).

During the course of microbial infection various adaptive strategies are employed by the immune system. One such strategy, and arguably the most important, is the production of an antibody response. Antibodies capable of binding antigens displayed by the infectious agent are produced and bind to and allow killing of the microorganism through complement activation, recruitment of macrophage and through direct interaction with the microbe itself. The therapeutic efficacy of antibodies capable of binding a given antigen varies and this is reflected in the fact that antibody production by the immune system matures during the course of an infection and becomes focussed in the case of a patient successfully fighting off an infection.

The antibody response is elicited by the B cell repertoire where individual B cells each produce structurally diverse antibody molecules. The actual size of this B cell/antibody repertoire is unknown, but it is estimated that the random clonal frequency of reactivity for a given antigen may be as high as 1 in 100,000 in cultured B cells (Nobrega, A., et al., Eur J Immunol. 1998 Apr;28(4):1204-15; PMID: 9565360). During the course of infection, antibodies capable of binding the pathogen are selected for by changes in the B cell population resulting in key antibodies being produced in large numbers. The mechanisms for these changes include clonal expansion, isotype switching, and somatic mutation of immunoglobulin variable regions. B cells responsible for generating antibodies which are able to bind a pathogen multiply, thus skewing the B cell repertoire and changing the proportions of B cells.

Antibody specific against the cell-binding domain of the *C.difficile* exotoxin A is found to be protective in a mouse model, and patients colonized with *C.difficile* but asymptotic for it are found to have an elevated anti-exotoxin A IgG titer. Infected patients developing such elevated serum anti-exotoxin A IgG titers in response to colonization are 48 times less likely to suffer from CDAD than patients who did not develop such an elevated titer. Therefore vaccination with *C.difficile* exotoxin A is suggested as a therapeutic strategy,

as is the use of anti-*C.difficile* exotoxin A antibodies (Giannasca PJ and Warny M., Vaccine, 2004 Feb 17;22(7): 848-56; PMID: 15040937).

Non-antibiotic based therapeutic regimes for the treatment/prevention of *C. difficile* infection are based upon vaccination and passive immunization. Vaccination treatment comprises administering to a patient either a nucleic acid sequence encoding an immunogenic fragment of the *C. difficile* surface layer protein or a variant or homologue thereof, or an equivalent polypeptide fragment (as disclosed in WO 02/062379). Passive immunotherapy is typically achieved by administering to a patient a monoclonal antibody specific to an immunogen produced by a pathogen. In general, passive immunotherapy is particularly effective in treating immunocompromised patients who are unable to respond to vaccination, and to patients who need immediate therapy and cannot wait for vaccination to take effect. In the case of a *C. difficile* infection, passive immunization relies on the administration to a patient of toxin-neutralizing polyclonal immune globulin, (as disclosed in WO 99/20304), or antibodies raised against the whole bacterium and the toxins (as disclosed in WO 96/07430).

Therefore the effective treatment of *C.difficile* infection is highly important, and the prior art teaches that the target for such therapy should be vaccination or passive immunotherapy targeted against the *C.difficile* exotoxin A, against the surface layer protein, or as a polyclonal serum specific against unidentified bacterial toxins.

However, the present inventor has identified a specific target for therapy. The experiments below show that patients infected with *C.difficile* produce an elevated titre of antibodies specific against acetyl-CoA acetyltransferase (thiolase), and that anti-acetyl-CoA acetyltransferase (thiolase) antibody *per se* is useful and effective in treating *C.difficile* infection. Furthermore, anti-acetyl-CoA acetyltransferase antibody, particularly a synthetic antibody constructed from the most dominant CDR sequences from *C.difficile* infected

patients, when used together with the antibiotics vancomycin or gentamicin, results in synergistic therapy of *C.difficile* infection.

Thus according to a first aspect of the present invention there is provided the use of an inhibitor of acetyl-CoA acetyltransferase in the manufacture of a medicament for the treatment or prophylaxis of infection by *Clostridium difficile*.

As used herein, the term "treatment" is intended to have a broad meaning unless explicitly stated otherwise. Thus by "treatment" or "therapy" is meant any treatment which is designed to cure, alleviate, remove or lessen the symptoms of, or prevent or reduce the possibility of contracting disorders or malfunctions of the human or animal body. Thus by the term "treatment" is meant both treatment of disease conditions, as well as their prophylaxis.

In particular, the acetyl-CoA acetyltransferase may be from *Clostridium difficile*, and it may have the sequence of SEQ ID NO: 43.

A wide range of inhibitors may be used which are specific against the acetyl-CoA acetyltransferase. In particular, the acetyl-CoA acetyltransferase may form a specific binding pair (sbp) with the inhibitor, the acetyl-CoA acetyltransferase being the first member of the pair, and the inhibitor being the second member.

Herein, a "Member of a Specific Binding Pair" is one of two different molecules, having an area on the surface or in a cavity which specifically binds to and is thereby defined as complementary with a particular spatial and polar organization of the other molecule. The members of the specific binding pair are referred to as ligand and receptor (antiligand), sbp member and sbp partner, sbp members or the like. These are usually members of an immunological pair such as antigen-antibody, although the term does have a broader

meaning encompassing other specific binding pairs, such as RNA-protein, DNA-protein, and the like.

Thus the inhibitor may be an antibody or an antigen binding fragment thereof, and it may be specific against an epitope displayed by the peptide of SEQ ID NO: 47

As detailed below in the Experiments section, the epitope displayed by the peptide having the sequence of SEQ ID NO: 47 has been identified as being conserved between various antigens and is therefore identified as being the peptide displaying the conserved epitope shared by those antigens.

The term "antibody" in its various grammatical forms is used herein to refer to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e. molecules that contain an antibody combining site or paratope. Such molecules are also referred to as "antigen binding fragments" of immunoglobulin molecules.

Illustrative antibody molecules are intact immunoglobulin molecules, substantially intact immunoglobulin molecules and those portions of an immunoglobulin molecule that contain the paratope, including those portions known in the art as Fab, Fab', F(ab')2, scFv and F(v). Antibodies, their production and use are well known in the art (e.g. Harlow, E. and Lane, D., "Using Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, New York, 1998).

As detailed in the experiments below, the variable heavy (VH) and variable light (VL) chains of antibodies produced by patients infected with *C.difficile* have been cloned and sequenced and their complementarity determining regions (CDRs) identified. This has shown that there are highly immunodominant VH chain CDRs 1-3 (CDR-H1, CDR-H2

and CDR-H3), and highly immunodominant VL chain CDRs 1-3 (CDR-L1, CDR-L2 and CDR-L3).

Thus, the antibody or antigen binding fragment thereof may have VH chain complementarity determining regions (CDRs) 1-3 having the sequences of SEQ ID NOs: 2-4 respectively. In particular, the antibody or antigen binding fragment thereof may have a VH chain having the sequence of SEQ ID NO: 1.

Similarly, the antibody or antigen binding fragment thereof may have VL chain complementarity determining regions (CDRs) 1-3 having the sequences of SEQ ID NOs: 17-19. In particular, the antibody or antigen binding fragment thereof may have a VL chain having the sequence of SEQ ID NO: 16.

A synthetic antibody having the above sequences has been created and used, and thus the antibody may have the sequence of SEQ ID NO: 41. The antibody detailed below having SEQ ID NO: 41 also comprises an N-terminal S-Tag and a C-terminal His-Tag. The tags are useful for experimental purposes but are unnecessary for the therapeutic antibody, although they (or other tags) may be considered useful. Thus the antibody may e.g. additionally comprise a His-Tag, for example a C-terminal His-Tag.

The experiments below have shown that not only is an inhibitor of acetyl-CoA acetyltransferase effective on its own in effecting treatment of *Clostridium difficile* infection, but it is also useful when used in conjunction with existing antibiotics, particularly gentamicin and vancomycin, and that such use can provide synergistic results. Preliminary experimental results also show that synergy is achieved with metronidazole.

Thus, the medicament may additionally comprise at least one of the group of antibiotics consisting: gentamicin, vancomycin and metronidazole.

Naturally, medical formulations of the present invention may comprise a pharmaceutically acceptable carrier, diluent or excipient (Remington's Pharmaceutical Sciences and US Pharmacopoeia, 1984, Mack Publishing Company, Easton, PA, USA; United States Pharmacopoeia, ISBN: 1889788031).

As detailed above, an inhibitor of acetyl-CoA acetyltransferase effective on its own in effecting treatment of *Clostridium difficile* infection. Thus also provided according to the present invention is isolated and/or purified antibody comprising at least one of the group consisting:

(i) VH chain complementarity determining regions (CDRs) 1-3 having the sequences of SEQ ID NOs: 2-4 respectively; and

(ii) VL chain complementarity determining regions (CDRs) 1-3 having the sequences of SEQ ID NOs: 17-19.

As above, the VH chain may have the sequence of SEQ ID NO: 1. The VL chain may have the sequence of SEQ ID NO: 16. The antibody may have the sequence of SEQ ID NO: 41.

Also provided is a nucleic acid molecule coding for such an antibody. The nucleic acid molecule may be isolated and/or purified. In particular, the nucleic acid molecule may have the sequence of SEQ ID NO: 46.

Also provided according to the present invention is the use of an inhibitor of acetyl-CoA acetyltransferase and vancomycin in the manufacture of a medicament for the prophylaxis or treatment of infection by *Enterococcus faecium*, or *Enterococcus faecalis*, particularly vancomycin resistant *Enterococcus faecium*.

The experiments below show that not only does the use of an inhibitor of acetyl-CoA acetyltransferase and vancomycin result in synergistic therapy of *C.difficile* infection, but

it also results in synergistic therapy of *E.faecium* infection, particularly vancomycin resistant *E.faecium* infection. Preliminary experiments have also shown that synergy is also observed in the treatment of *E.faecalis* infection.

The acetyl-CoA acetyltransferase may be from *Clostridium difficile*.

The inhibitor may be an antibody or an antigen binding fragment thereof, and it may be specific against an epitope displayed by the peptide having the sequence of SEQ ID NO: 47.

As with other aspects of the present invention, the antibody or antigen binding fragment thereof may have VH chain complementarity determining regions (CDRs) 1-3 having the sequences of SEQ ID NOs: 2-4 respectively. The antibody or antigen binding fragment thereof VH chain may have the sequence of SEQ ID NO: 1.

The antibody or antigen binding fragment thereof may have VL chain complementarity determining regions (CDRs) 1-3 having the sequences of SEQ ID NOs: 17-19. The antibody or antigen binding fragment thereof VL chain may have the sequence of SEQ ID NO: 16.

The antibody or antigen binding fragment thereof may have the sequence of SEQ ID NO: 41.

Where the present invention is concerned with the provision of medicaments containing two or more active ingredients (for example antibody and an antibiotic), the present invention also provides combined preparations comprising such active ingredients. For example, two-pack preparations may be provided.

Thus also provided according to the present invention is a combined preparation for the treatment of infection by *Clostridium difficile*, comprising:

(i) an inhibitor of acetyl-CoA acetyltransferase; and

(ii) at least one of the group of antibiotics consisting: gentamicin, vancomycin, and metronidazole.

Also provided is a combined preparation for the treatment of infection by *Enterococcus faecium* or *Enterococcus faecalis*, comprising:

(i) an inhibitor of acetyl-CoA acetyltransferase; and

(ii) vancomycin.

The *Enterococcus faecium* or *Enterococcus faecalis* may be vancomycin resistant.

The present invention also extends to methods of treatment of patients. Thus also provided according to the present invention is a method of treatment of infection by *Clostridium difficile*, comprising administering a therapeutically effective quantity of an inhibitor of acetyl-CoA acetyltransferase to a patient in need of same. The method may additionally comprise administering a therapeutically effective quantity of at least one of the group of antibiotics consisting: gentamicin, vancomycin and metronidazole.

Also provided according to the present invention is a method of treatment of infection by *Enterococcus faecium* or *Enterococcus faecalis*, comprising administering a therapeutically effective quantity of:

(i) an inhibitor of acetyl-CoA acetyltransferase; and

(ii) vancomycin

to a patient in need of same.

The *Enterococcus faecium* may be vancomycin resistant.

The invention will be further apparent from the following description, which show, by way of example only, forms of treatment of *C.difficile* and *E.faecium* infection.

Experiments

In the experiments below, a synthetic antibody has been constructed using the most predominant VH and VL antibody sequences from patients infected with *C.difficile*. Using this synthetic antibody, an antigen was isolated and purified and electrospray mass spectrometry used to determine a putative partial sequence for the isolated protein. Searching of *C.difficile* genomic sequences for matches identified a possible candidate matching sequence. A comparison of the candidate *C.difficile* sequence identified a number of homologous proteins which are classified as acetyl-CoA acetyltransferase (thiolase) enzymes and confirms it as a member of that family. The closest match is NP_349376 from *Clostridium acetobutylicum*, which showed a 68% homology over 391 amino acids. Cloning, expression and purification of the candidate protein gave a protein which bound strongly with the synthetic antibody. Further experiments have shown that the antibody specific against the *C.difficile* acetyl-CoA acetyltransferase (thiolase) shows synergy with vancomycin and gentamicin in treating *C.difficile* infections. Preliminary experiments also show that synergy can also be achieved with metronidazole.

Experiments also show synergy between the synthetic antibody and vancomycin in the treatment of vancomycin resistant *E.faecium*. Preliminary experiments (not shown) also indicate synergy between the synthetic antibody and vancomycin in the treatment of *E.faecalis* infection.

Unless stated otherwise, all procedures were performed using standard protocols and following manufacturer's instructions where applicable. Standard protocols for various techniques including PCR, molecular cloning, manipulation and sequencing, the manufacture of antibodies, epitope mapping and mimotope design, cell culturing and phage display, are described in texts such as McPherson, M.J. *et al.* (1991, PCR: A practical approach, Oxford University Press, Oxford), Sambrook, J. *et al.* (1989, Molecular

cloning: a laboratory manual, Cold Spring Harbour Laboratory, New York), Huynh and Davies (1985, "DNA Cloning Vol I - A Practical Approach", IRL Press, Oxford, Ed. D.M. Glover), Sanger, F. *et al.* (1977, PNAS USA 74(12): 5463-5467), Harlow, E. and Lane, D. ("Using Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, New York, 1998), Jung, G. and Beck-Sickinger, A.G. (1992, Angew. Chem. Int. Ed. Eng., 31: 367-486), Harris, M.A. and Rae, I.F. ("General Techniques of Cell Culture", 1997, Cambridge University Press, ISBN 0521 573645), "Phage Display of Peptides and Proteins: A Laboratory Manual" (Eds. Kay, B.K., Winter, J., and McCafferty, J., Academic Press Inc., 1996, ISBN 0-12-402380-0).

Reagents and equipment useful in, amongst others, the methods detailed herein are available from the likes of Amersham (www.amersham.co.uk), Boehringer Mannheim (www.boehringer-ingeltheim.com), Clontech (www.clontech.com), Genosys (www.genosys.com), Millipore (www.millipore.com), Novagen (www.novagen.com), Perkin Elmer (www.perkinelmer.com), Pharmacia (www.pharmacia.com), Promega (www.promega.com), Qiagen (www.qiagen.com), Sigma (www.sigma-aldrich.com) and Stratagene (www.stratagene.com).

The contents of each of the references discussed herein, including the references cited therein, are herein incorporated by reference in their entirety.

Where "PMID:" reference numbers are given for publications, these are the PubMed identification numbers allocated to them by the US National Library of Medicine, from which full bibliographic information and abstract for each publication is available at www.ncbi.nlm.nih.gov. This can also provide direct access to electronic copies of the complete publications, particularly in the case of e.g. PNAS, JBC and MBC publications.

Sequence homology is as determined using the BLAST2 program (Tatusova TA *et al.*, FEMS Microbiol Lett. 1999 May 15;174(2):247-50; PMID: 10339815) at the National Center for Biotechnology Information, USA (www.ncbi.nlm.nih.gov) with default parameters.

Identifying dominant VH and VL antibody sequences from patients infected with *C.difficile*

In order to identify antibody sequences from patients with *C.difficile* infections and determine the dominant antibody sequences, particularly CDR sequences, the methods detailed in WO 03/052416 were used. B cells producing antibodies were identified, sequenced of and analysed, using the following basic steps (see WO 03/052416):

(1) Isolation of VH and/or VL coding regions from circulatory B cells of human patients.

(2) Determining the Nucleotide sequence of VH and/or VL repertoires.

(3) Determination of VH and/or VL primary amino acid sequences.

(4) Extraction of CDR regions *in silico* - incorporation into the database

(5) Detection of dominant CDR & framework regions in VH and/or VL repertoire.

(6) Construction and Production of therapeutic recombinant antibodies from dominant antibody sequences.

Fragments of antibodies were sequenced from four infected patients (D01, D02, D03 and D04).

Heavy Chain (CDH1)

CDH1 was the most commonly occurring VH chain from the *C.difficile* infected patients. It was identified from analysis of the CDR3 sequences from patient antibody VH chains. 226/1011 (22.4%) of cloned antibodies from *C.difficile* infected patients had the same

CDR3 sequence (CDR-H3, below). CDH1 was found to be present in three quarters of patients. Its CDR3 sequence appeared in 184/318 (57.9%) of the clones from patient D01; in D03 it appeared in 40/291 (13.7%) of the clones; and in D04 it appeared in 2/252 (0.8%) of the clones. The most common full VH sequence is SEQ ID NO: 1.

Within this VH chain sequence, the CDR (complementarity determining region) sequences are as follows:

CDR-H1	SEQ ID NO: 2
CDR-H2	SEQ ID NO: 3
CDR-H3	SEQ ID NO: 4

Homology searches have identified several other CDR3 sequences with greater than 70% homology to the CDR-H3 sequence (SEQ ID NO: 4), all from *C.difficile* patients. The CDR3 sequences are SEQ ID NOs: 5-15.

Light Chain (CDL1)

The light chain for H1L1 was derived from a clone with the most common CDR3 sequence from *C.difficile* infected patients. CDL1 was present in patient D01 at a frequency of 84/251 (33.5%). The most common VL sequence containing this CDR3 sequence was SEQ ID NO: 16.

Within this VL chain sequence, the CDR (complementarity determining region) sequences are as follows:

CDR-L1	SEQ ID NO: 17
CDR-L2	SEQ ID NO: 18
CDR-L3	SEQ ID NO: 19

Homology searches have identified several other CDR3 sequences with greater than 70% homology to the CDR-L3 sequence, all from *C.difficile* patients. The CDR3 sequences are SEQ ID NOs: 20-40.

Construction and Cloning of Antibody H1L1

The synthetic antibody H1L1 was constructed as follows: CDH1 and CDL1 were separately PCR amplified from the sequencing vector and cloned into cloning vector pGEM-T easy (Promega Corporation) to facilitate DNA sequencing. For this 3 μg PCR product was prepared for restriction using QIAquick PCR purification spin columns (Qiagen, UK) according to the manufacturers instructions. DNA was eluted from the spin column in 40 μL buffer EB. Purified PCR product (2 μL) was mixed with 1 μL pGEM-T easy vector, 6 μL water and 1 μL DNA ligase and the mixture ligated for 1 h at room temperature. Ligations were then transformed into electrocompetent *E. coli* TG1 cells (Stratagene) by electroporation, and plated out onto agar plates containing Ampicillin 100 μg ml^{-1} IPTG (100 μM) and X-gal (0.006 % w/v). Colonies were allowed to grow overnight at 37 °C and then stored at 4 °C. Recombinant colonies are identified as white colonies on this media.

This gave an antibody having the general structure:

S Tag - CDH1 - Linker - CDL1 - His Tag

with the amino acid sequence of SEQ ID NO: 41 with an N-terminal S-Tag and a C-terminal His-Tag.

Identification of the target for Antibody H1L1 - Acetyl-CoA acetyltransferase (Thiolase)

Materials and methods

Sample preparation:

Clostridium difficile cells (NCTC 11204) grown on blood agar plates were suspended in 10 mM of PBS (phosphate buffered saline) and sonicated for 5 x 1 minute on ice. The cell lysate was centrifuged at 13000 rpm for 5 minutes and 300 µl of the supernatant was precipitated in 20 mL 10 % trichloroacetic acid and 20 mM DTT (dithiothreitol) in cold acetone for 45 minutes. The proteins were recovered by centrifugation and the pellet was washed three times with cold acetone containing 20 mM DTT.

2D-gel electrophoresis:

The protein pellet was dissolved in sample rehydration solution (7 M urea, 2 M thiourea, 3% (w/v) CHAPS, 0.002% bromophenol blue in water) and diluted with the same solution for isoelectric focusing. Isoelectric focusing was performed using a Zoom IPGRunner (RTM) System (Invitrogen Ltd, Carlsbad, CA, USA) over a non-linear pH range of 3-10 (7 cm) for a total of 1700 Vh, loading approximately 15 µg of protein on each strip. Prior to the second dimension separation the strips were equilibrated for 15 minutes in equilibration buffer (50 mM Tris-HCl pH 8.8, 6 M urea, 30% glycerol, 2 % (w/v) SDS, 0.002% bromophenol blue in water) containing 65 mM DTT and then in the same buffer containing 125 mM iodoacetamide for another 15 minutes.

The second dimension separation was carried out using NuPage 4-12 % Bis-TrisZoom gels and MOPS buffer (Invitrogen Ltd, Carlsbad, CA, USA). The proteins were transblotted onto Invitrolon PVDF membrane (Invitrogen Ltd, Carlsbad, CA, USA) and blocked in 5% skimmed milk in 0.1% Tween20 in 10 mM PBS for 1 hour.

Immunoblotting:

To identify the target of antibody H1L1, the membrane was incubated for 1 hour with purified H1L1 antibody in 5% skimmed milk in 0.1% Tween20 in 10 mM PBS. After washing, an anti-His antibody conjugated with horse radish peroxidase (Santa Cruz

Biotechnologies) was used, diluted at a ratio of 1:500 with 0.1% Tween20 in 10 mM PBS, to detect bound H1L1. After washing with 0.1% Tween20 in 10 mM PBS the blot was developed using ECL (Amersham biosciences, Little Chalfont, UK).

To visualise targets for IgG antibodies in patients infected with *Clostridium difficile*, membranes were incubated for 1 hour with serum diluted 1:20 - 1:50 with 0.1% Tween20 in 10 mM PBS. Anti IgG-antibody conjugated with horse radish peroxidase (for ECL) or alkaline phosphatase (Sigma) where the membrane was developed using SigmaFast (RTM) 5-bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium tablets (Sigma) was used.

Electrospray-mass spectrometry:

Protein bands or spots cut out from the gel were washed in 25 mM ammonium bicarbonate for 10 minutes and dehydrated using 25 mM ammonium bicarbonate:acetonitrile 1:2 for 15 minutes. After repeating the wash with ammonium bicarbonate and the dehydration step, the gel pieces were dried down in a SpeedVac (RTM). Small volumes of trypsin (10 µg/ml in 25 mM ammonium bicarbonate) were added until gel pieces returned to their original size and a small amount of 25 mM ammonium bicarbonate was added to keep the gel pieces wet during digestion. The samples were digested for 4.5 hours at 37 °C and the peptides extracted for 15 minutes by adding 80 µl acetonitrile. The supernatant was dried in a SpeedVac until all the acetonitrile had evaporated. The sample was desalted using C-18 Zip-Tips (Millipore) and analysed using nano-spray time-of-flight mass spectrometry (Q-TOF, Micromass, Manchester, UK)

Results:

Isolation of target for antibody H1L1 using 2D gels and immunoblotting.

2D gels combined with immunoblotting were used to pinpoint the antigen on the 2D gel. Two blots where compared, one using the serum from the patient whose most abundant antibody was the template for H1L1 and one using the expressed and purified H1L1. Both

reacted to the same protein spot. The same protein was antigenic in four patients who had recovered from diarrhoea due to *C. difficile*.

Isolation of target protein using a column method

A clinical isolate of *C.difficile* (referred to herein as strain CD 14000287, isolated from a *C.difficile* infected patient) was grown anaerobically in brain heart infusion broth supplemented with thioglycolic acid and L-cysteine-HCl for 48 hours at 37 °C. The cell suspension was centrifuged in a Sorval RC-3B centrifuge at 5000 rpm for 20 minutes to pellet the cells and the cell pellet was washed once with PBS and re-centrifuged as above. The washed cell pellet was resuspended in PBS and stored at -20 °C until further use. A 2 ml aliquot of the frozen cell suspension was thawed and centrifuged at 14000 rpm for 5 minutes on a benchtop microfuge to pellet the cells, and the pelleted cells were resuspended in 4 ml of 6 M guanidine-HCl pH 8.0. The guanidine extracted protein was clarified by centrifugation at 45000 rpm on a Beckman L8-70M ultracentrifuge for 1 hour at 20 °C. The supernatant was dialysed at room temperature against three times 2 litres of 20 mM sodium carbonate buffer pH 9.5 to exchange the extracted protein into a compatible buffer for the AminoLink (supplied by Perbio) column. 2 ml of an H1L1 antibody solution (~2 mg/ml) in 20 mM sodium carbonate pH 9.5 was covalently coupled to the AminoLink resin equilibrated with 20 mM sodium carbonate pH 9.5 in a prepacked 2 ml column following the manufacturer's instructions (Perbio). The *C.difficile* antigen containing extract (1.5 ml) was then applied to the covalently coupled H1L1 column and incubated with the resin for 1 hour at room temperature. Unbound material was washed through the column with 12 ml of 20 mM sodium carbonate buffer pH 9.5 and the bound antigen eluted by applying 8 ml of 0.1 M glycine buffer pH 2.5-3.0. The eluted protein was neutralised with 1 M Tris pH 9.0 prior to concentration on an Amicon 15 ml concentrator adaptor to ~200 µl. This concentrated protein material was then analysed by 10 % (w/v) SDS-PAGE and visualised by Coomassie Blue staining. The H1L1 interacting protein band was then identified by mass spectrometry.

Identification of isolated protein using mass spectrometry

The protein band or spot was cut out of the gel and digested at 37 °C with trypsin. The samples were analysed using nano electrospray time-of-flight mass spectrometry, which gave the sequence of SEQ ID NO: 42.

The same peptide was found in the samples from the 2D gel experiments as from the protein isolated using the column based method.

Searching the *Clostridium difficile* genome using BLAST search at the Sanger Institute (www.sanger.ac.uk/projects/C_difficile/) gives SEQ ID NO: 43 as a match.

Theoretical pI: 5.74 Molecular weight: 43430.30 Da which correlates well with the location of the protein on the 2D gel where the molecular weight was approximately 44 kD and pI 5.5-6.

Using the protein sequence of SEQ ID NO: 43 in a protein-protein NCBI BLAST search at www.ncbi.nlm.nih.gov/BLAST/ using default settings reveals high similarity to acetyl-CoA-acetyltransferase (thiolase) accession number NP_349376 with 68% identity over 391 amino acids.

Cloning of the acetyl-CoA acetyltransferase

For cloning and expression in *E.coli* the sequence encoding the acetyl-CoA acetyltransferase was PCR amplified directly from *C. difficile* genomic DNA, and prepared using DNeasy spin columns (Qiagen) according to the manufacturer's instructions.

PCR primers used were identified and designed by direct comparison with the *C.difficile* genomic DNA sequence encoding the candidate match for the acetyl-CoA acetyltransferase, and are SEQ ID NOs: 44 and 45, synthesized by SIGMA Genosys.

Amplification was carried out using *Taq* DNA polymerase (Invitrogen) allowing direct ligation-independent cloning into the expression vector pBAD-TA (Invitrogen), adding a C-terminally fused His_6-tag to the expressed acetyl-CoA acetyltransferase under the control of the Arabinose-inducible promoter *ara*BAD. The cloning mix was transformed in to the expression strain TOP10 (Invitrogen) and recombinants were identified using SDS-PAGE and immunoblotting using a monoclonal anti-His-tag peroxidase-conjugate antibody (SIGMA). The resulting plasmid is referred to herein as pThioII.

Expression and purification of the acetyl-CoA acetyltransferase

For over-expression of the 6-His-tag thiolase fusion protein, *E.coli* strain TOP10 (pThioII) was grown to late exponential phase (OD_{600} 1.0 at 37 °C with shaking at 200 rpm) and protein expression was induced by the addition of Arabinose to a final concentration of 0.2%. After a further 240 minutes growth, bacteria were harvested by centrifugation (500 g, 10 minutes 4 °C) and the pellet was resuspended in lysis buffer (6 M Guanidine Hydrochloride, 50 mM Tris-HCl pH 8) at 1/20 the starting volume and solubilized by mixing for 1 hour at room temperature. Insoluble material was removed by a further centrifugation step (10000 g, 10 minutes at room temperature) and the supernatant applied to a Qiagen spin-prep Ni-NTA column following the manufacturer's instructions. The column was washed three times with 600 µl of lysis buffer followed by three washes with 600 µl of wash buffer 1 (8 M Urea, 50 mM Tris/HCl pH 8) and three times with 600 µl of wash buffer 2 (8 M Urea, 50 mM TrisHCl pH 8, 25 mM Imidazole). The His-Tag protein was eluted from the column with 100 µl of elution buffer (8 M Urea, 50 mM Tris/HCl pH 8, 250 mM Imidazole), the elution step was repeated and both fractions pooled. Samples were analysed by SDS-PAGE.

Testing Antibody H1L1 against cloned acetyl-CoA acetyl transferase

Cloned and purified acetyl-CoA acetyl transferase was mixed 1:1 with loading buffer and DTT and put on heating block at 90 °C for 5 minutes. 5-15 µl was separated for 35 minutes on a 10% BisTris gel (Invitrogen) using NuPAGE-MES SDS running buffer (Invitrogen).

The proteins were transblotted onto Invitrolon PVDF membrane (Invitrogen Ltd, Carlsbad, CA, USA) and blocked in 5% skimmed milk in 0.1% Tween20 in 10 mM PBS for 1 hour. The membrane was incubated with H1L1 at 1:10 (concentration approximately 400 µg/ml) in 0.1% Tween20 in 10 mM PBS for 1 hour. Washed three times for 5 minutes with 0.1% Tween20 in 10 mM PBS and then incubated for one hour with S-protein HRP conjugate (Novagen) at 1:1000. The blot was developed using Sigma-Fast DAB stain and shows a strong response to the thiolase band located just under the 50 kD marker.

Therefore, antibody from patients with *C.difficile* infection is focussed against *C.difficile* acetyl-CoA acetyl transferase and synthetic antibody constructed from the dominant light and heavy chain CDRs of those patients is specific against the *C.difficile* acetyl-CoA acetyl transferase. Therefore, *C.difficile* acetyl-CoA acetyl transferase is a strong antigen and antibody H1L1 is useful in binding it.

Conserved epitope

There are three consecutive enzymes in the butanol/butyrate-producing pathway of *Clostridium acetobutylicum* which are also present on the *Clostridium difficile* chromosome. These have been found to react with a rabbit anti-serum raised against supernatant proteins from *Clostridium difficile* (Mullany P *et al.*, FEMS Microbiol Lett., 1994 Nov 15;124(1): 61-7; PMID: 8001771).

Assuming the enzymes share a conserved epitope, the only homologous region can be found at residue 9-12 in thiolase (accession number P45362) which is present in crotonase (accession nuber P45361) residues 5-8, and this conserved sequence is SEQ ID NO: 47.

***Enterococcus faecium* and *Clostridium difficile* combination drug MIC**

Pre-Assay Preparation

The organisms were cultured onto a Columbia Blood Agar plate (CBA) to achieve single colonies and incubated at 37 °C for 24 hours (*E.faecium*) or anaerobically for 48 hours (*C.difficile*).

Antimicrobial agents were prepared according to NCCLS methodology (M7-6A), giving a total of 14 concentrations for vancomycin and gentamicin and 11 concentrations for H1L1. The initial dilutions of vancomycin and gentamicin were made in dH_2O, H1L1 was initially diluted in Formulation Buffer (In 100 ml: 3.484 g Arginine (200 mM), 3.006 g Urea (0.5 M), pH 9.5). Subsequent dilutions were made in the appropriate growth medium for the organism being used. The agents were stored in aliquots at -20 °C and thawed on the day of use.

Concentrations were twice the final concentration required.

1. Medium Preparation

E.faecium Medium - Cation-adjusted Mueller Hinton Broth (NCCLS M7-6A): To 1 litre Mueller Hinton Broth (OXOID, MHB), 2 ml of 10 mg/ml $CaCl_2$ and 500 µl of 10 mg/ml $MgCl_2$ were added

C.difficile Medium -

(1) Supplemented Brucella Broth (NCCLS M11-A5): To 900 ml Brucella Broth powder (SIGMA), 1 ml Hemin (5 mg/ml) and 1 ml Vitamin K (1 mg/ml) were added. Post autoclaving 100 ml Lysed horse blood (5%) was added.

(2) Reinforced clostridial medium (RCM) was prepared according to the manufacturers instructions: 38 g in 1 litre dH_2O.

2. MIC Plate Preparation – Table 1

Drug 1 - vancomycin or gentamicin (final concentration range 0.0625 µg/ml to 512 µg/ml)

In a U-shaped 96 well microtitre plate: twice the required concentration of vancomycin or gentamicin (50 µl) is added left to right along the first row of the microtitre plate leaving the final well blank. This was repeated for the other rows using 2-fold decreasing concentrations of vancomycin.

Drug 2 - H1L1 antibody (final concentration range 0.25 µg/ml to 256 µg/ml)

H1L1 (50 ml) is added down the columns. Twice the required concentration is added down the first column. This was repeated for the other columns moving along the plate (left to right) 2-fold decreasing concentrations of H1L1. The last column is left blank

The final column contains 100 µl of growth medium only

Continuing concentrations of vancomycin and gentamicin were performed in the same manner as above but on a second microtitre plate. H1L1 concentrations were exactly the same as above, as were the Media only control in column 12.

Inoculum Preparation - Direct Colony Suspension

- The inoculum was prepared immediately prior to use of the bacteria.
- A direct colony suspension was made by resuspending colonies from an 18 to 24 hour (*E.faecium*) or 48 hour (*C.difficile*) agar plate into the appropriate growth medium or sterile saline.
- This was adjusted to 0.5 MacFarlands standard then diluted 1:10 in growth medium (approximately 1 x 10^7 cfu/ml) according to NCCLS M7-6A (*E.faecium*) and NCCLS M11-A5 (*C.difficile*).

Plate Inoculation

- 5 µl of the 1:10 inoculum suspension prepared as above was used to inoculate each well (final inoculum 5 x 10^4 cfu/ml).
- The plate was inoculated from well 12 to well 1.

Incubation

- The plates were incubated at 37 °C for 24 hours (*E.faecium*) or anaerobically for 48 hours (*C.difficile*).
- To check the inoculum 10 µl from the growth control was diluted in 10 ml sterile saline (1:1000), and 100 µl was plated onto a CBA plate and incubated at 37 °C for 24 hours (*E.faecium*) or anaerobically for 48 hours (*C.difficile*). Fifty colonies were equivalent to 5 x 10^4 cfu/ml.

Reading Results

The MIC was taken as the lowest concentration of drug that substantially inhibited the growth of the organism.

The FIC (fractional inhibitory concentration) was calculated for each drug by dividing the MIC in the presence of the second drug by the MIC in its absence. For each combination this produced two fractions, which were summated to produce the FICI (fractional inhibitory concentration index): synergy was defined by a value of <=0.5, indifference was defined by a value of >0.5 to <4 and antagonism was defined by a value of >= 4.0

Results

These results demonstrate synergy between H1L1 and vancomycin and gentamicin

Conclusions

These results demonstrated synergy between both (i) H1L1 and vancomycin, and (ii) H1L1 and gentamicin versus *C. difficile* 14000287 and *C.difficile* NCTC 11204. It showed synergy between Vancomycin and H1L1 in Vancomycin resistant *Enterococcus faecium*.

Growth of *Clostridium difficile* in the presence of variable short chain fatty acids (SCFA) and H1L1 antibody concentrations

SCFA Preparation

The three SCFAs used were acetate, propionate and butyrate.

Each was prepared to a concentration of 1 M as follows:

Sodium acetate FW 82.03 - 82.03 g in 1 litre dH_2O

Sodium butyrate FW 110.09 - 110.09 g in 1 litre dH_2O

Sodium propionate FW 96.06 - 96.06 g in 1 litre dH_2O

The three solutions were combined in a ratio of 70:20:10 respectively. Therefore, in the final solution the concentrations were sodium acetate 0.7 M, sodium butyrate 0.2 M and sodium propionate 0.1 M. The final concentration of total SCFA was 1 M.

From this stock solution concentrations of 5, 10, 20, 30, 40 and 50 mM total SCFA were prepared using reinforced clostridial medium (RCM, DIFCO).

Medium preparation

Reinforced clostridial medium (RCM) was prepared according to the manufacturers instructions: 38 g in 1 litre dH_2O

Growth Curve

Day before assay

Cultures were only removed from anaerobic conditions immediately prior to use. Exposure to aerobic conditions did not exceed 30 minutes

An overnight suspension of *C.difficile* was made by inoculation 10 ml of RCM medium with colonies from a 48 hour old Columbia Blood Agar plate of the organism. The suspension was incubated for 24 hours at 37 °C under anaerobic conditions.

Day of assay

Control plate

In a flat-bottomed 96 well microtitre plate control solutions (200 µl) were placed in the wells as outlined below in Table 4. Each control used 3 wells.

Test plate

In a flat-bottomed 96 well microtitre plate (rows labelled A-H, columns labelled 1-12) test solutions (100 µl) were placed in the wells as detailed below. The two test solutions, SCFA plus H1L1, give a combined volume of 200 µl. Each test used 2 wells. Wells in row

A contained 50 mM SCFA; in row B, 40 mM SCFA; in row C, 30 mM SCFA; in row D, 20 mM SCFA; in row E, 10 mM SCFA; in row F, 0.5 mM SCFA. Wells in columns 1 and 2 contained 16 μg/ml H1L1; in columns 3 and 4, 8 μg/ml H1L1; in columns 5 and 6, 4 μg/ml H1L1; in columns 7 and 8, 2 μg/ml H1L1; in columns 9 and 10, 1 μg/ml H1L1; in columns 11 and 12, 0.5 μg/ml H1L1. Row G was empty. 200 μl RCM was placed into all the wells in row H as a growth control for the plate.

Inoculum preparation

Cultures were only removed from anaerobic conditions immediately prior to use. Exposure to aerobic conditions did not exceed 30 minutes.

The overnight suspension, culture at late log phase, was used to inoculate the microtitre plates. 20 μl (approx 10% inoculum) was added to all the wells, except for the media/negative control on the control plate.

Plates were incubated at 37 °C under anaerobic conditions for 24 hours.

Optical density readings (OD_{600} nm) were made at time points zero and 24 hours. Average OD_{600} nm readings were determined.

Results

Controls - see Table 5.

Conclusions -

1. No effect of 10, 20, 30, and 40 mM of SCFA on its own, but some decrease with 50 mM of SCFA.
2. No effect with 0.5, 1, 2 4, and 8 μg/ml of H1L1 but inhibition with 16 μg/ml of H1L1.

Tests - see Table 6.

Conclusions -

Indifference with 1, 2 and 4 μg/ml of H1L1 and 5 mM of SCFA (data not shown). Synergy with 8 μg/ml of H1L1 and SCFA from 10-50 mM with optical density at 24 hours falling to a range of 0.296 - 0.330. The effect was most obvious with 16 μg/ml of H1L1 and 50 mM of SCFA.

Table 1

Drug concentrations at this stage will be half the concentration that was put into the wells (50 µl + 50 µl = 1:2 dilution).

A, B, etc. = Vancomycin or gentamicin

1, 2, 3 etc. = + H1L1

	1	2	3	4	5	6	7	8	9	10	11	12
A	A1	A2	A3	A4	A5	A6	A7	A8	A9	A10	A11	Media only
B	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10	B11	Media only
C	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11	Media only
D	D1	D3	D4	D4	D5	D6	D7	D8	D9	D10	D11	Media only
E	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	Media only
F	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	F11	Media only
G	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	Media only
H	H1	H2	H3	H4	H5	H6	H7	H8	H9	H10	H11	Media only

Table 2

Species	Agent	MIC (μg/ml) of each agent		FIC (μg/ml)	FICI	Outcome
		Alone	Combination			
Clostridium difficile NCR 1104 (Brucella broth)	Vancomycin	1	0.125	0.125	0.125	Synergy
	H1L1	512	0.25	0.0004		
Enterococcus faecium	Vancomycin	128	16	0.12	0.37	Synergy
	H1L1	512	128	0.25		

Table 3

Species	Agent	MIC (μg/ml) of each agent		FIC (μg/ml)	FICI	Outcome
		Alone	Combination			
C.difficile 14000287 (Brucella broth)	Gent	8	2	0.25	0.266	Synergy
	H1L1	512	8	0.0156		
C.difficile NCTC 11204 (RCM)	Gent	16	4	0.25	0.375	Synergy
	H1L1	512	64	0.125		

Table 4

	1	2	3	4	5	6	7	8	9	10	11	12
A	RCM no inoculum			RCM + inoculum								
B	RCM + 50 mM SCFA			RCM + 40 mM SCFA			RCM + 30 mM SCFA			RCM + 20 mM SCFA		
C	RCM + 10 mM SCFA			RCM + 5 mM SCFA								
D	RCM + 16 µg/ml H1L1			RCM + 8 µg/ml H1L1			RCM + 4 µg/ml H1L1			RCM + 2 µg/ml H1L1		
E	RCM + 1 µg/ml H1L1			RCM + 0.5 µg/ml H1L1								
F												
G												
H												

Table 5

Experiment No	H1L1 (µg/ml)	SCFA (mM)	Mean Optical Density at 24 Hours
1	-	-	0.452
2	-	5	0.460
3	-	10	0.429
4	-	20	0.452
5	-	30	0.480
6	-	40	0.440
7	-	50	0.370
8	0.5	-	0.450
9	1	-	0.440
10	2	-	0.440
11	4	-	0.440
12	8	-	0.420
13	16	-	0.334

Table 6

Experiment No	H1L1 (µg/ml)	SCFA (mM)	Mean Optical Density at 24 Hours
1	8	10	0.310
2	16	10	0.308
3	8	20	0.296
4	16	20	0.228
5	8	30	0.305
6	16	30	0.308
7	8	40	0.310
8	16	40	0.303
9	8	50	0.330
10	16	50	0.281

CLAIMS

1. The use of an inhibitor of acetyl-CoA acetyltransferase in the manufacture of a medicament for the treatment or prophylaxis of infection by *Clostridium difficile,* wherein the inhibitor is an antibody or an antigen binding fragment thereof.

2. The use according to claim 1, said acetyl-CoA acetyltransferase being from *Clostridium difficile.*

3. The use according to claim 1, said antibody or antigen binding fragment thereof being specific against an epitope displayed by the peptide having the sequence of SEQ ID NO: 47.

4. The use according to either of claims 1 or 3, said antibody or antigen binding fragment thereof having VH chain complementarity determining regions (CDRs) 1-3 having the sequences of SEQ ID NOs: 2-4 respectively.

5. The use according to claim 4, said antibody or antigen binding fragment thereof VH chain having the sequence of SEQ ID NO: 1.

6. The use according to any of claims 4-5, said antibody or antigen binding fragment thereof having VL chain complementarity determining regions (CDRs) 1-3 having the sequences of SEQ ID NOs: 17-19.

7. The use according to claim 6, said antibody or antigen binding fragment thereof VL chain having the sequence of SEQ ID NO: 16.

8. The use according to claim 1, said antibody or antigen binding fragment thereof having the sequence of SEQ ID NO: 41.

9. The use according to any of the preceding claims, the medicament additionally comprising at least one of the group of antibiotics consisting: gentamicin, vancomycin and metronidazole.

10. A combined preparation for the treatment of infection by *Clostridium difficile*, comprising:

(i) an inhibitor of acetyl-CoA acetyltransferase wherein the inhibitor is an antibody or an antigen binding fragment thereof; and

(ii) at least one of the group of antibiotics consisting: gentamicin, vancomycin and metronidazole.

11. The use of an inhibitor of acetyl-CoA acetyltransferase and vancomycin in the manufacture of a medicament for the prophylaxis or treatment of infection by *Enterococcus faecium* and *Enterococcus faecalis*, wherein the inhibitor of acetyl-CoA acetyltransferase is an antibody or an antigen binding fragment thereof.

12. The use according to claim 11, said *Enterococcus faecium* or *Enterococcus faecalis* being vancomycin resistant.

13. The use according to either of claims 11 or 12, said acetyl-CoA acetyltransferase being from *Clostridium difficile*.

14. The use according to claim 11, said antibody or antigen binding fragment thereof being specific against an epitope displayed by the peptide having the sequence of SEQ ID NO: 47.

15. The use according to either of claims 11 or 14, said antibody or antigen binding fragment thereof having VH chain complementarity determining regions (CDRs) 1-3 having the sequences of SEQ ID NOs: 2-4 respectively.

16. The use according to claim 15, said antibody or antigen binding fragment thereof VH chain having the sequence of SEQ ID NO: 1.

17. The use according to any of claims 11 or 14 to 16, said antibody or antigen binding fragment thereof having VL chain complementarity determining regions (CDR) 3 having the sequence of SEQ ID NO: 19.

18. The use according to claim 17, said antibody or antigen binding fragment thereof VL chain having the sequence of SEQ ID NO: 16.

19. The use according to claim 11, said antibody or antigen binding fragment thereof having the sequence of SEQ ID NO: 41.

20. A combined preparation for the treatment of infection by *Enterococcus faecium* or *Enterococcus faecalis*, comprising:

(i) an inhibitor of acetyl-CoA acetyltransferase wherein the inhibitor is an antibody or an antigen binding fragment thereof; and

(ii) vancomycin.