Specification

A novel antibiotic comprising an antibody mimetic, preparation methods and uses thereof

FIELD OF THE INVENTION

The present invention belongs to the domain of biology and medicine, and especially relates to a novel antibiotic comprising an antibody mimetic, its preparation methods and uses thereof.

RELATED ART

Since Penicillin and other antibiotics were brought into use in 1944, Diplococcus meningitides, and other life-threatening pathogenic bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Neisseria meningitidis* have acquired drug resistance. According to relative reports published by United States Disease Control Center (CDC) in recent years, these antibiotics would be likely to lose effectiveness completely in 10 or 20 years.

The present antibiotics kill pathogenic bacteria by restraining synthesis of cell wall, restraining or interfering the pathway of bacterial nucleic acid and protein metabolism and synthesis. However, these antibiosis methods are more likely to lead to drug resistance resulted by bacteria mutation. Therefore scientists are dedicated to develop novel antibiotics. One of the comparatively promising directions is to imitate the inter-killing mechanism among homogeneous heterologous strains so as to develop novel antibiotics. In Nature, a number of bacterial toxins can directly form ion channels on bacteria cytomembrane to kill bacteria. The typical specimen is a kind of bacterial toxin secreted by *E.col*, colicin. One of which is Colicin Ia, found in 1952 by Jacob. After efforts of generations, the transmembrane spatial structure of Colicin Ia when ion channels open as well as shut on the artificial lipide bimolecular film (Qiu et al, Major transmembrane movement associated with Colicin Ia channel gating. J. Gen. Physiology, 107: 313-328 (1996)) was disclosed in 1996. This established theoretical laid the basis for designing and preparing novel antibiotics on molecular level.

As mentioned above, colicin is a kind of ideal ion channel antibiotic model, but wild-type colicin can only act on homogeneous heterologous strains. We must change the targeting of the colicin so that they are capable of acting on other pathogenic bacteria. Porin, a kind of pore protein existing on the outer membrane of bacteria, mitochondria or chloroplast, allows bigger molecules to pass through. Porin also has a higher immunogenicity, and can induce high level expression of monoclonal antibody in host cells. It should be an ideal development direction for antibody research, if we can design an antibody mimetic with better recognizing ability to change the targeting of the colicin, by using the antibody specific for porins on outer membran of bacteria as the antetype of the antibody mimetic.

SUMMARY OF THE INVENTION

To overcome the above technical defects and make up a deficiency in the art, the present invention provides a novel antibiotic. Its antibacterial ability is a thousand-fold more powerful than regular antibiotics. Due to its unique action mechanism, drug resistance resulted in mutation can hardly be acquired by pathogenic bacteria. And the antibiotic will not hurt normal human cells when it kills pathogenic bacteria.

A novel antibiotic comprising an antibody mimetic and a colicin,
or comprising an antibody mimetic and a channel-forming domain of a colicin,
the antibody mimetic covalently bonded to the carboxyl end of the polypeptide of the colicin or the channel-forming domain of the colicin, wherein the colicin is selected from a group consisting of colicin E1, Ia, Ib, A, B, N; wherein said antibody mimetic being yielded by fusing two complementarity determining regions (CDRs), V_HCDR_1 and V_LCDR through a cognate framework region (V_HFR_2) of an immunoglobulin; wherein the immunoglobulin specifically recognizes bacterium porins.

Wherein the bacterium is *Neisseria meningitides*, the porin is PorA in the outer membrane of *Neisseria meningitidis* cells.

Wherein the immunoglobulin consist of two covalent bonded polypeptides with PUBMED Accession 2MPA_H and PUBMED Accession 2MPA_L.

Wherein the colicin is Ia.

A peptide molecule, comprising an antibody mimetic and a colicin,
or comprising an antibody mimetic and a channel-forming domain of a colicin,
the antibody mimetic covalently bonded to the carboxyl end of the polypeptide of the colicin or the channel-forming domain of the colicin, wherein the colicin is selected from a group consisting of colicin E1, Ia, Ib, A, B, N; wherein said antibody mimetic being yielded by fusing two complementarity determining regions (CDRs), V_HCDR_1 and V_LCDR3 through a cognate framework region (V_HFR_2) of an immunoglobulin; wherein the immunoglobulin specifically recognizes bacterium porins.

One of the foresaid peptide molecules, its amino acid sequence set forth in SEQ ID NO.6.

A nucleic acid molecule encoding any one of foresaid peptide molecules.

Wherein the nucleic acid molecule with nucleotide sequence set forth in SEQ ID NO.5.

A recombinant plasmids containing any one of foresaid nucleic acid molecules.

A preparation method of any one of foresaid novel antibiotics, one of foresaid recombinant plasmids is transfected into an expression system, and the novel antibiotic is separated and purified from expressed polypeptide.

Any one of foresaid novel antibiotics used for preparing antibacterial medicament.

Wherein the antibacterial medicament is used for killing *Neisseria meningitidis*, vancomycin-resistant *Enterococcus faecalis*, methicillin-resistant *Staphylococcus aureus* or multidrug-resistance *Pseudomonas aeruginosa.*.

The novel antibiotics of this invention are based on the foundation of colicin's characteristic of

forming ion channels on the target pathogen membrane, which causes the pathogen to leak out its content and die. The targeting structure is an antibody mimetic with some domains of an immunoglobulin specifically recognizing porin protein on target pathogen. The antibody mimetic comprises two complementarity determining regions (CDRs), V_HCDR_1 and V_LCDR3, and a cognate framework region (V_HFR_2) of an immunoglobulin; the three regions covalently form a linear peptide molecule as V_HCDR1-V_HFR2-V_LCDR3 from amino end to carboxyl end. It is well known that the active regions of an immunoglobulin for recognition reaction are called complementary determining region that has only about several to a dozen of amino acids. The antibody mimetic in the novel antibiotic, compared with a nature antibody molecule or any present artificial reconstructed antibody such as seFv and Fab, has smaller molecular weight, nicer tissue penetration and simpler structure without most parts of frame structure and Fc fragment of a nature antibody. So it is be able to reduce immunoreaction in patients and easily guides colicin contained in the novel antibiotic to infected tissues and identifies the pathogenic bacteria. In clinical application, the novel antibiotic is directed to membrane of the target pathogenic bacteria by antibody mimetic contained, and the colicin contained forms ion channels on bacteria cytomembrane of the target bacteria and kills the target bacteria by leading to leak-out of its cytoplasm. The antibacterial ability of the novel antibiotic also applies to bacterial strains with drug resistance. The recognition sites are unique antigenic properties on bacterial surface, and not located on human cytomembrane, so the novel antibiotic is safe for human. Compared to other antibiotics that easily cause drug resistance, the antibiotic in this invention kills the pathogenic bacteria not by the porin but by the colicin acting on biomembrane of the pathogenic bacteria and forming ion channels to make the cell leaking out the cytoplasm to die. The antibody mimetic for targeting just needs to guide the colicin to the pathogenic bacteria. Bacteria's drug resistance is normally acquired by changing porin's structure and creating barrier for antibiotic's entry. The antibiotic in this invention only requires porin's antibody recognition sites for purpose of killing the pathogenic bacteria. The novel antibiotics identify the sites of porin on the membrane of bacteria, but the colicin of the novel antibiotic binds on other sites and forms ion channels to make the bacteria to leak out to die. The action sites are not the porin. So the pathogenic bacteria is not likely to acquire drug resistance to the novel antibiotic by mutating, evoluting, throwing away or changing the structure of porin which is necessary for survival. According to this inventive concept, the novel antibiotic has many variants due to the diversity of porin on bacterium surface and the diversity of the immunoglobulin recognizing the

porins.

Since the meningitis caused by *Neisseria meningitidis* severe threatens infants and children's health, the drug resistance of *Neisseria meningitidis* to common medicine is very serious. The dosage is higher and higher in order to inhibit the bacteria efficiently, which seriously endangers the health of patients. Therefore, on the basis of foresaid inventive concept of this invention, the inventor reconstructed and obtained an antibody mimetic of an antibody which was specific for the porin A of *Neisseria meningitidis*. The heavy chain peptide of the antibody had an accession number: 2MPA_H at PubMed Home. The light chain peptide had an accession number: 2MPA_L at PubMed Home. The antibody mimetic, of which the amino acid sequence set forth in Seq ID No.2, was connected on the C-terminus of the Colicin Ia's peptide to constitute a novel antibiotic PMC-AM1 with amino acid sequence set forth in Seq ID No.6. As survival curve of mice shown in Fig.7, the survival rate of the mice injected with fatal dose of *Neisseria meningitides* in PMC-AM1 group was 90% in 8 days. It showed that the antibacterial activity and protective effect in vivo of the novel antibiotics are superior to current normal antibiotics such as penicillin and gentamicin. Meanwhile a comparative test on bactericidal effect was set to compare the minimum inhibitory concentration (MIC value) of PMC-AM1, ceftazidime and ampicillin for *Neisseria meningitidis*. As shown in Fig.6A, the MIC value of PMC-AM1 was 0.11nMol, ceftazidime was 3.02nMol and ampicillin was 1.35nMol. This result indicated that the bactericidal ability of PMC-AM1 is significantly more powerful than antibiotics currently used for inhibiting *Neisseria meningitidis*.

In present invention, some experiments were set to test the bactericidal effect of the novel antibiotic PMC-AM1 on other pathogens with serious drug-resistance. The result showed the PMC-AM1 had extremely stronger antibacterial ability on multi-drug resistant *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus faecalis*, methicillin-resistant *Staphylococcus aureus*, as shown in Fig.5, its antibacterial ability on multi-drug resistant *Pseudomonas aeruginosa*, was 127 to 3800 times stronger than that of ceftazidime, levofloxacin, gentamicin etc. And as shown in Fig. 6B and Fig. 6C, PMC-AM1 has obvious inhibition effect on vancomycin-resistant *Enterococcus faecalis*, methicillin-resistant *Staphylococcus aureus*.

The novel antibiotic of this invention can be used to prepare antibacterial drugs, especially for

Neisseria meningitidis, *Pseudomonas aeruginosa*, vancomycin-resistant *Enterococcus faecalis* or methicillin-resistant *Staphylococcus aureus* .

The nucleotide sequences encoding the peptides of the novel antibiotics can be cloned into the expression vector to construct recombinant plasmids, which express fusion protein in host cell. The novel antibiotic protein is isolated from expressed fusion protein.

According to degeneracy of nucleotide codons, the nucleotide sencoding antibiotic of the invention is adjustable. One of skill in the art would be able to adjust the nucleotide sequence according to the host cells' preference to the nucleotide codon. As long as the encoded polypeptide has no change, the nucleotide sequences are still in the scope of inventive concept of this invention.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig.1 shows the structure of recombinant plasmid comprising the gene of antibody mimetic and gene of Colicin Ia, referred to herein as pBHC-PorA1.

The peptide of the antibody mimetic bonded to the C-terminus of Colicin Ia, and the amino acid sequence of the antibody mimetic is set forth in Seq ID No.2.

Fig.2 shows the structure of the recombinant plasmid comprising the gene of antibody mimetic and gene of Colicin Ia, referred to herein as pBHC-PorA2.

The peptide of antibody mimetic is linked to the C-terminus of Ia's peptide, and the amino acid sequence of the antibody mimetic set forth in Seq ID No.4. In the antibody mimetic, a V_HCDR1 and a reversed V_LCDR3 are connected through a cognate framework region (V_HFR_2).

Fig.3 illustrates the construction of the novel antibiotic.

In which, T and R are signal recognition domains on the N-terminus of a Colicin Ia. The channel forming, a structure domain being capable of forming ion channels, is situated at C-terminus of the Colicin Ia. The AM is an antibody mimetic.

Fig.4 shows the result of experiment for inhibiting ability of the novel antibiotic PMC-AM1 to *Diplococcus intracellularis.*

In the curve, from left to right shows the control, 5μg/ml Ampicillin, 5μg/ml PMC-AM2, 5μg/ml PMC-AM1, 10μg /ml PMC-AM1.

The horizontal ordinate shows growth time of bacteria, the unit is hours; the longitudinal ordinate shows optical density of the bacteria medium at 600 nm, indicating the quantity of bacteria growth.

Fig.5 shows the minimum inhibitory concentration value（MIC）of the novel antibiotic tested by Agar dilution method.

The plates shows the MIC of the drugs to multi-drug resistant *Pseudomonas aeruginosa:* Con was blank control, (A) the MIC of ceftazidime was 16 μg / ml, (B) the MIC of levofloxacin was 8 μg / ml, (C) the MIC of gentamicin was greater than 512 μg / ml, (D) the MIC of PMC-AM1 was 8 μg / ml.

Fig.6 shows the comparison experiment of minimum inhibitory concentration of the novel antibiotic of this invention with the commonly used antibiotics to methicillin-resistant *Staphylococcus aureus* (ATCC BAA-42), vancomycin-resistant *Enterococcus faecalis* (ATCC 700802), multi-drug resistant *Pseudomonas aeruginosa* (isolated by West China Hospital, No.13578) and *Nesseria meningitidis* (No.29332 of bacteria Preservation Center in China）.

In which，the longitudinal ordinate shows minimum inhibitory concentration (nMol);

A shows the result of *Nesseria meningitidi*: (1) PMC-AM1, MIC = 0.11 nMol, (2) ceftazidime, MIC = 3.02 nMol, (3) ampicillin, MIC = 1.35 nMol;

B shows the result of vancomycin-resistant *Enterococcus faecalis* (1) PMC-AM1, MIC = 0.23 nMol, (2) vancomycin, MIC = 21.54 nMol, (3) ampicillin, MIC = 10.78 nMol;

C shows the result for methicillin-resistant *Staphylococcus aureus*:(1) PMC-AM1, MIC = 0.06 nMol, (2) ampicillin, MIC = 21.55 nMol, (3) oxacillin, MIC =14.1 nMol;

D shows the result for multi-drug resistant *Pseudomonas aeruginosa*: (1) PMC-AM1, MIC=0.91nMol, (2) levofloxacin, MIC=43.2 nMol, (3) ceftazidime, MIC=29.3 nMol, (4) gentamicin, MIC>889.4 nMol.

Fig.7 shows survival curve of comparison of inhibition effective of the novel antibiotic, the wild-type colicin and the polypeptide anti-*Staphylococcal aureus* disclosed in China patent ZL 01128836.1 on methicillin-resistant *Staphylococcus aureus* (ATCC BAA-42), vancomycin-resistant *Enterococcus faecalis* (ATCC 700802), and multi-drug resistant *Pseudomonas aeruginosa* (isolated by West China Hospital, No.13578).

In which, longitudinal ordinate shows minimum inhibitory concentration(nMol);

A shows the result for vancomycin-resistant *Enterococcus faecalis*: (1) the polypeptide anti-*Staphylococcus aureus*, MIC = 0.91nMol, (2) the wild-type Colicin Ia, MIC =0.91 nMol, (3) PMC-AM1, MIC = 0.23 nMol;

B shows the result for methicillin-resistant *Staphylococcus aureus*: (1) the polypeptide anti-*Staphylococcus aureus*, MIC = 0.06nMol, (2) the wild-type Colicin Ia, MIC =0.23 nMol, (3) PMC-AM1, MIC = 0.06 nMol.

C shows the result for multidrug resistance *Pseudomonas aeruginosa* : (1) the polypeptide anti-*Staphylococcus aureus*, MIC = 0.91 nMol, (2) the wild-type Colicin Ia, MIC =0.91 nMol, (3) PMC-AM1,MIC=0.23 nMol.

Fig.8 shows survival curves, which were the result of in vivo experiment of the novel antibiotics to protect animals infected by *Nesseria meningitidis*.

In which, horizontal ordinate shows the survival time of mice, days; Longitudinal ordinate shows the number of animal survival. 1) PMC-AM1; 2) Gen was gentamicin; 3) PEN was penicillin; 4) Con. was blank control. The injection concentration of all test drugs is by 1.5mg/kg (drug's weight/ mouse's weight).

EMBODIMENTS

The invention is further illustrated by the following embodiments as well as the drawings.

Embodiment 1: Construction of plasmids expressing the novel antibiotics and preparation of the novel antibiotics

The original plasmid was pSELECT^{TM}-1 plasmid (8.3kb) with genes of Colicin Ia and immunity protein. By Double Strands Oligo nucleotide Point Mutation Technology (QuickChange^{TM} Kit, Strategene corporation) the gene encoding the antibody mimetic set forth in SEQ ID NO. 1 or 3 were inserted to 626 amino acid position of Colicin Ia gene. Two recombinant plasmids, herein referred to as pBHC-PorA1 and pBHC-PorA2 shown in Fig.1 and Fig. 2, were constructed, which were used to prepare the novel antibiotics. The recombinant plasmids were respectively transfected into *E.coli* BL-21 engineering bacterium to express the novel antibiotics.

The mutation procedure was preceded according to the manual of Strategene Quick Change Site Directed Mutagenesis Kit (catalog #200518),

1. Point mutation reactant was prepared as follows:

5μl 10X buffer

2μl (10ng) original plasmid pSELECT^{TM}-1 with genes of Colicin Ia and immunity protein.

1.25μl (125ng) artificial 5'-3' oligo nucleotide primer (refer to below oligo nucleotide primer)

1.25μl (125ng) artificial 3'-5' oligo nucleotide primer (refer to below oligo nucleotide primer)

1μl dNTP

50μl de-ionized water

1μl Pfu

(The above drugs were all reagents in the medical kit, except plasmid, primer and de-ionized water.)

2. PCR amplification was proceeded with the amplification conditions as follow: denaturalize at 95 ℃ for 35 seconds, anneal at 53℃ for 70 seconds, extend at 68℃ for 17 minutes, totally 20 cycles.

3. 1μl endonuclease Dpn1 was incorporated to digest parent DNA chain(37℃, 1h); 1μl digestion product was placed on ice and incubated with 50μl XL1-Blue competent cells for 30 minutes, heat shock at 42℃ for 45 seconds, and then taken into ice for 2 minutes;

4. 0.5ml NZY culture medium was added, the bacteria solution (reactant of step 3, i.e. transformed cells from competent cells) was shaken at 220 rpm and 37℃ for 1 hour; then 50-100 μl reactant was taken out to plank on medium plate (LB culture medium with 1% agar and 50μg/ml ampicillin, at 37℃ over night);

5. The bacteria was picked out after cultivating 18 hours, the plasmid was abstracted and sequenced to ascertain it had successful mutation;

6. 100ng mutated plasmid was placed on ice and incubated with 40 μl of BL-21 competent cells for 5.minutes, heat shock at 42℃ for 30 seconds, and then placed on ice for 2 minutes. 160 μl SOC culture medium was added; bacteria was shaken at 220 rpm, 37℃ for 1 hour and taken out to plank on medium plate (LB culture medium with 1% agar, 50μg/ml ampicillin, at 37℃ cultivating one night); monoclone colonies are picked out for largely reproducing;

7. The bacteria was largely reproduced in 8-10L FB culture medium, at 250 rpm, 30℃ for 3-4 hours, and warmed to 42℃ at 250 rpm for 0.5hours and then cooled to 37□at 250 rpm for 1.5hours. The thallus was centrifugated at 4℃, 6000g for 20 minutes, and then was suspended in 80-100 ml of 50mM boric acid buffer fluid (pH 9.0, with 2mM EDTA) at 4℃. After being added in 50μg PMSF the thallus was ultrasonicated at 4℃, 400W for 1 minute and repeated 4-5 times with 2-3 minutes interval for maintaining the temperature of the bacteria solution. The cracked thallus was

high-speed centrifugated at 4℃, 75,000g for 90 minutes. The 5,000,000 unit streptomycin sulfate was added into the supernatant to deposit DNA (stiring at 4℃ for 1 hour). After centrifugated at 10000g, 4℃ for 10 minutes, the supernatant was loaded in bag filter of 15,000 molecular weight and dialysed by 10L of 50 mM boric acid buffer fluid over night at 4℃; then centrifugated again at 10000g , 4℃ for 10 minutes. The supernatant was loaded on CM ion-exchange column. Then the CM ion-exchange column was washed thoroughly and the novel antibiotic was eluted by 0.3 M NaCl+50 mM boric acid buffer fluid. Corresponding to the above two kinds of recombinant plasmid, the novel antibiotics were named PMC-AM1 和 PMC-AM2 of which the amino acid sequences were set forth in Seq ID No.6 and Seq ID No.8 respectively.

The AM1 was a peptide chain comprising of the peptides of the first complementarity determining domain in variable region of the heavy chain, the peptide of the second frame region of the heavy chain and the peptide of the third complementarity determining domain in variable region of the light chain. Its amino acid sequence was set forth in Seq ID No.2. The AM2 was a peptide chain comprising of the peptide of the first complementarity determining domain in variable region of the heavy chain, the peptide of the second frame region of the heavy chain and the peptide of reversed third complementarity determining domain in variable region of the light chain. Its amino acid sequence was set forth in Seq ID No.4.

The PMC-AM2 was constructed as a control of the PMC-AM1 for testing the activity of the novel antibiotics of present invention when the domains composing the antibody mimetic were connected in different order.

The artificial oligo nucleotide sequences for preparing above two mutation plasmids respectively are as follow:

5'—3' (SEQ ID NO.9)

gcg aat aag ttc tgg ggt att *TCT TAT TGG CTG CAT TGG ATT AAA CAG* taa ata aaa tat aag aca ggc

3'—5' (SEQ ID NO.10)

gcc tgt ctt ata ttt tat tta *CTG TTT AAT CCA ATG CAG CCA ATA AGA* aat acc cca gaa ctt att cgc

5'—3' (SEQ ID NO.11)

tgg ctg cat tgg att aaa cag *AGA CCT GGT CAG GGA CTG TGG ATC GGA* taa ata aaa tat aag aca ggc

3'—5' (SEQ ID NO.12)

gcc tgt ctt ata ttt tat tta *TCC GAT CCA CAG TCC CTG ACC AGG TCT* ctg ttt aat cca atg cag cca

5'—3' (SEQ ID NO.13)

ggt cag gga ctg tgg atc gga *TCT CAG TCC ACG CAT GTG CCG AGA ACC* taa ata aaa tat aag aca ggc

3'—5' (SEQ ID NO.14)

gcc tgt ctt ata ttt tat tta *GGT TCT CGG CAC ATG CGT GGA CTG AGA* tcc gat cca cag tcc ctg acc

pBHC-PorA 2

5'—3' (SEQ ID NO.15)

gcg aat aag ttc tgg ggt att *TCT TAT TGG CTG CAT TGG ATT AAA CAG* taa ata aaa tat aag aca ggc

3'—5' (SEQ ID NO.16)

gcc tgt ctt ata ttt tat tta *CTG TTT AAT CCA ATG CAG CCA ATA AGA* aat acc cca gaa ctt att cgc

5'—3' (SEQ ID NO.17)

tgg ctg cat tgg att aaa cag *AGA CCT GGT CAG GGA CTG TGG ATC GGA* taa ata aaa tat aag aca ggc

3'—5' (SEQ ID NO.18)

gcc tgt ctt ata ttt tat tta *TCC GAT CCA CAG TCC CTG ACC AGG TCT* ctg ttt aat cca atg cag cca

5'—3' (SEQ ID NO.19)

ggt cag gga ctg tgg atc gga *ACC AGA CCG GTG CAT ACG TCC CAG TCT* taa ata aaa tat aag aca ggc

3'—5' (SEQ ID NO.20)

gcc tgt ctt ata ttt tat tta *AGA CTG GGA CGT ATG CAC CGG TCT GGT* tcc gat cca cag tcc ctg acc.

Embodiment 2: Inhibiting effect of the novel antibiotic on *Nesseria meningitidis* .

The bacteria was strain No. 29332 *Nesseria meningitidis* of Bacteria Preservation Center in China), two microliters (μl) of bacteria solution (10^5CFU/ml) was added in 10ml rabbit blood-chocolate medium (containing 50mg beef extract, 100mg tryptone, 50mg NaCl, 30 mg K_2HPO_4 and 0.5-0.8 ml off fiber rabbit blood). Five groups were prepared. The first group as control was added 0.3 M NaCl + 50 mM boric acid buffer fluid (i.e. blank preservative fluid for the novel antibiotic, by the same volume as the novel antibiotic solution in the experimental groups). Penicillin sodium was added in the second group by 5μg/ml. The novel antibiotic PMC-AM1 was added in the third group by 5μg/ml. The novel antibiotic PMC-AM2 was added in the forth group by 5μg/ml. The novel antibiotic PMC-AM1 was added in the fifth group by 10μg/ml.

Reactant liquid of the above five groups were respectively put into 100ml conical flask, and cultured at 37℃ by 200rpm. 100μl culture solution was sampled per hour and added onto 96-pore ELISA plate for measuring bacterial turbidity by spectrophotometer (A595nm) color comparison. The bacteria-growth curve was drawn up to compare the bacteriostatic efficacy of the novel antibiosis. The result, as shown in Fig.4, showed that *Nesseria meningitidis* could only be restrained by PMC-AM1.

Embodiment 3. Contrast experiment of the minimum inhibitory concentration of the novel antibiotic and normal antibiotics on multi-drug resistant *Pseudomonas aeruginosa.*

Testing the minimum inhibitory concentration（MIC）of the novel antibiotic by the agar dilution method. The bacteria were inoculated on the surface of agar plate containing different concentrations of drugs by multipoint inoculate instrument (Deneley A400). The bacteria concentration on per point was 10^5 CFU/ml. After incubated at 37℃ for 18-24 hours, the result can be observed. The least concentration of drugs in the plating medium without bacteria growth was Minimum Inhibitory Concentration (MIC) of the drug to the said bacteria.

Experimental strain was multi-drug resistant *Pseudomonas aeruginosa* which was a clinical isolated strain (isolated by West China Hospital, No.13578) using MH medium（per 100ml containing 500mg beef extract, 1.75g casein acid hydrolysate, 150mg soluble starch and 1.7g agar）.

As the result showed in Fig.5, the MIC of the novel antibiotic (D) PMC-AM1 on multi-drug resistant *Pseudomonas aeruginosa* was 8 μg/ml, ceftazidime (A) was 16 μg/ml, levofloxacin (B) was 8 μg/ml, and gentamicin (C) was greater than 512 μg/ml. If in terms of molecular weight standard, the MIC of PMC-AM1 on multi-drug resistant *Pseudomonas aeruginosa* was 0.23 nMol, ceftazidime (A) was 29.3 nMol, levofloxacin was 43.2 nMol, and gentamicin(C) was greater than 890 nMol, i.e. the antibacterial effect of PMC-AM1 on multi-drug resistant *Pseudomonas aeruginosa* was stronger 127-3800 times than ceftazidime, levofloxacin and gentamicin.

Embodiment 4. Contrast experiment of the antibacterial activity in vitro between the novel antibiotic of this invention and normal antibiotics.

Testing the minimum inhibitory concentration（MIC）of the novel antibiotic by the agar dilution method. The bacteria were inoculated on the surface of agar plate containing different

concentrations of drugs by multipoint inoculate instrument (Deneley A400). The bacteria concentration per point was 10^5CFU/ml. After incubated at 37□ for 18-24 hours, the result can be observed. The least concentration of drugs in the plating medium without bacteria growth was Minimum Inhibitory Concentration (MIC) of the drug to the said bacteria.

Experimental strains were multi-drug resistant *Pseudomonas aeruginosa* which is a clinical isolated strain (isolated by West China Hospital, No.13578) using MH medium (Per 100ml containing 500mg beef extract and 1.75g_casein acid hydrolyzate, 150mg soluble starch, 1.7g_gelose), methicillin-resistant *Staphylococcus aureus* (ATCC BAA-42) using BM medium (Per 100ml containing 1g tryptone, 0.5g yeast powder, 0.1g glucose, 100mg KH_2PO_4, 1g NaCL and 1g_agar), vancomycin-resistant *Enterococcus faecalis* (ATCC 700802) using the MH medium; *Nesseria meningitidis* (No.29332 of Bacteria Preservation Center in China) using the same medium used in the_embodiment 2 (in addition added Columbia blood agar base 3.9g).

The result shown in Fig.6, A showed the result of *Nesseria meningitidis*: (1) PMC-AM1, MIC = 0.11nMol, (2) ceftazidime, MIC=3.02 nMol, (3) ampicillin, MIC =1.35 nMol. B showed the result of vancomycin-resistant *Enterococcus faecalis*: (1) PMC-AM1, MIC=0.23 nMol, (2) vancomycin, MIC=21.54 nMol, (3) ampicillin, MIC=10.78 nMol. C showed the result for methicillin-resistant *Staphylococcus aureus:* (1) PMC-AM1, MIC=0.06nMol, (2) ampicillin MIC=21.55nMol, (3) oxacillin, MIC =14.1 nMol. D showed the result for multi-drug resistant *Pseudomonas aeruginosa*: (1) PMC-AM1, MIC=0.91nMol, (2) levofloxacin, MIC=43.2 nMol, (3) ceftazidime, MIC=29.3 nMol (4) gentamicin, MIC>889.4 nMol.

Embodiment 5. Contrast experiments of the antibacterial activity in vitro between the novel antibiotic of this invention, the polypeptide anti-*Staphylococcal aureus* and wild-type Colicin Ia.

Testing the minimum inhibitory concentration（MIC）of the novel antibiotic by the agar dilution method. The least concentration of drugs in the plating medium without bacteria growth was Minimum Inhibitory Concentration (MIC) of the drug to the said bacteria.

Experimental strains were multi-drug resistant *Pseudomonas aeruginosa (*isolated by West China Hospital, No.13578),methicillin-resistant *Staphylococcus aureus* (ATCC BAA-42), vancomycin-resistant *Enterococcus faecalis* (ATCC 700802), using MH medium； *Nesseria meningitidis* (No.29332 of bacteria Preservation Center in China).

The results shown in Fig.7, A showed the result for vancomycin-resistant *Enterococcus faecalis* : (1)the polypeptide anti-*Staphylococcal aureus*, MIC = 0.91nMol, (2) the wildtype Colicin Ia, MIC =0.91 nMol, (3) PMC-AM1,MIC = 0.23 nMol. B showed the result for methicillin-resistant *Staphylococcus aureus*: (1) the polypeptide anti-*Staphylococcal aureus*, MIC = 0.06nMol, (2) the wildtype Colicin Ia, MIC=0.23 nMol, (3) PMC-AM1, MIC=0.06nMol. C showed the result for multidrug resistance *Pseudomonas aeruginosa*:(1)the polypeptide anti-*Staphylococcal aureus*, MIC = 0.91nMol, (2) the wildtype Colicin Ia, MIC =0.91 nMol, (3) PMC-AM1, MIC = 0.23 nMol

Embodiment 6. In vivo protection experiments of the novel antibiotics for animals infected by *Nesseria meningitidis*.

Experimental materials

Drugs: PMC-AM1, gentamicin, ampicillin.

Experimental bacteria

Nesseria meningitides (No.29332 of Bacteria Preservation Center in China, i.e Center of Medical Devices of National Institute for the Control of Pharmaceutical & biological Products, SDA).

Experimental methods

As shown in Fig.7, 40 mice were divided into four experimental groups, 10 mice in each group. The mice were given an intraperitoneal injection of glucose solution of ferrous by 20mg/kg, and 1 hour later an intraperitoneal injection of 0.5 ml of bacteria culture containing 1 share of *Nesseria meningitidis*cultrue solution (its CFU was 2.36 x 10^9/ml) and 1.5 share of 5% dry yeast solution. One hour later after being given intraperitoneal injection of fatal dose of the bacteria culture, the mice in experimental group were given an intravenous injection of the drugs and the mice in control group were given an intravenous injection of normal saline, all drugs were injected by1.5mg/kg), observation every 2 hours for 8 days, the death of mice as the positive results.

In the Fig.7, 1.PMC-AM1 means the novel antibiotic obtained in this invention; 2. Gen means gentamicin; 3. PEN means penicillin; 4. Con. means control.

Results.

As the survive curve shown in Fig.7, after being given intraperitoneal injection of fatal dose of *Neisseria meningitides* solution, 1). Mice in the control group all died in two days, 2). Mice in penicillin group all died in two days, 3). Mice in gentamicin group had 50%survival rate in 8 days,

4).Mice in PMC-AM1 group had 90% survival rate in 8 days.

The result indicates the novel antibiotic PMC-AM1 of the present invention performed superior protection activity in vivo on mice infected by fatal dose of *Nesseria meningitidis* than common antibiotics.

Claims

1. A novel antibiotic comprising an antibody mimetic and a colicin,

or comprising an antibody mimetic and a channel-forming domain of a colicin,

the antibody mimetic covalently bonded to the carboxyl end of the polypeptide of the colicin or the channel-forming domain of the colicin, wherein the colicin is selected from a group consisting of colicin E1, Ia, Ib, A, B, N; wherein said antibody mimetic being yielded by fusing two complementarity determining regions (CDRs), V_HCDR_1 and V_LCDR through a cognate framework region (V_HFR_2) of an immunoglobulin; wherein the immunoglobulin specifically recognizes bacterium porins.

2. The novel antibiotic of claim 1, wherein the bacterium is *Neisseria meningitides*, the porin is PorA in the outer membrane of *Neisseria meningitidis* cells.

3. The novel antibiotic of claim 2, wherein the immunoglobulin consist of covalent bonded polypeptide with PUBMED Accession 2MPA_H and a polypeptide with PUBMED Accession 2MPA_L.

4. The novel antibiotic of claim 1 or claim 2 or claim 3, wherein the colicin is Ia.

5. A peptide molecule comprising an antibody mimetic and a colicin,

or comprising an antibody mimetic and a channel-forming domain of a colicin,

the antibody mimetic covalently bonded to the carboxyl end of the polypeptide of the colicin or the channel-forming domain of the colicin,, wherein the colicin is selected from the group consisting of colicin E1, Ia, Ib, A, B, N; wherein said antibody mimetic being yielded by fusing two complementarity determining regions (CDRs), V_HCDR_1 and V_LCDR3 through a cognate framework region (V_HFR_2) of an immunoglobulin; wherein the immunoglobulin specifically recognizes bacterium porins.,

6. One of the fusion peptide molecules of claim 5, its amino acid sequence sets forth in SEQ ID NO.6

7. A nucleic acid molecule encoding any fusion peptide molecule claimed in claim 5 or claim 6.

8.The nucleic acid molecule of claim 7, its nucleotide sequence sets forth in SEQ ID NO.5.

9. A recombinant plasmids containing any nucleic acid molecule of claim 7 or 8.

10. A preparation method of any one of the novel antibiotics claimed in claim1~4, one of the recombinant plasmids of claim 9 is transfected into an expression system, and the novel antibiotic is separated and purified from expressed polypeptide.

11. A use of any one novel antibiotics of claims 1~4 for preparing antibacterial medicament.

12. The use of claim 11, wherein the antibacterial medicament is used for killing *Neisseria meningitidis*, vancomycin-resistant *Enterococcus faecalis*, methicillin-resistant *Staphylococcus aureus* or multidrug-resistance *Pseudomonas aeruginosa.*

SEQUENCE LISTING

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<120> A novel antibiotic comprising an antibody mimetic, preparation methods and uses thereof

<130> P10581/JJQ

<160> 20

<170> PatentIn version 3.3

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Gln Gln Thr Arg Asn Asp Arg Ala Ile Ser Glu Ala Arg Asn Lys Leu
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