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ANTIBACTERIAL COMPOSITION AGAINST DRUG-RESISTANT BACTERIA OR PRO-INFLAMMATORY BACTERIA

Abstract

An antibacterial composition against drug-resistant bacteria or pro-inflammatory bacteria is disclosed. The antibacterial composition contains an intestinal bacterium as an active ingredient. Also uses of such composition useful for treating, ameliorating, or preventing an infectious disease or an inflammatory disease, and a pharmaceutical composition containing an intestinal bacterium as an active ingredient are disclosed.

Related U.S. Application Data

Publication Classification

Background/Summary

CROSS REFERENCE TO RELATED APPLICATIONS [0001] This application is a Divisional of U.S. application Ser. No. 17/436,148, filed on Sep. 3, 2021, which is a National Stage of International Application No. PCT/JP2020/009423, filed on Mar. 5, 2020, which claims priority from U.S. Provisional Application No. 62/815,101, filed on Mar. 7, 2019, the contents of all of which are incorporated herein by reference in their entireties.

INCORPORATION BY REFERENCE OF SEQUENCE LISTING
[0002] The instant application contains a Sequence Listing which has been filed electronically in xml format and is hereby incorporated by reference in its entirety. Said xml copy, created on Apr. 16, 2025, is named Q306447.xml and is 506,119 bytes in size.
TECHNICAL FIELD
[0003] The present invention relates to an antibacterial composition against drug-resistant bacteria or pro-inflammatory bacteria. The present invention also relates to a pharmaceutical composition or method for treating, ameliorating, or preventing diseases caused by drug-resistant bacteria or pro-inflammatory bacteria.
BACKGROUND ART
[0004] Various commensal bacteria are resident on mucous membranes such as the digestive tract and the oral cavity, forming a flora as a whole. The resident flora plays a very important role in the physiology and health maintenance of the host. A compositional abnormality of the resident flora is called dysbiosis, and has been gradually revealed to be a cause of various diseases. The advancement of the elucidation of the resident mucosal flora may have a high possibility of developing new disease countermeasures and treatments for various diseases, but the detailed mechanism of such flora has not been sufficiently clarified due to its complexity.
[0005] Humans produce and swallow about 1.5 liters of saliva every day. Normally, bacteria contained in saliva (oral bacteria) simply pass through the intestinal tract and do not colonize. However, in some situations, oral bacteria may colonize in the intestinal tract. Particularly in Crohn's disease, liver cirrhosis, and colorectal cancer, it has been reported that colonization of oral bacteria in the intestinal tract was observed even at the early stages of disease onset. In addition, it is known that the colonized oral bacteria affect the pathological conditions of the diseases (NPLs 1 to 6).
[0006] Meanwhile, by allowing oral bacteria of patients with Crohn's disease and the like to colonize in the intestinal tract to induce Th1 cells, the present inventors succeeded in isolating, culturing, and identifying the bacteria involved in the onset of the diseases (PTL 1). More specifically, the present inventors found that as a result of oral administration of saliva derived from a patient with Crohn's disease to germ-free mice, interferon gamma (IFN-γ)-producing CD4-positive T-cells (Th1 cells) significantly increased in the large intestine. Then, the present inventors succeeded in isolating and culturing the Kp2H7 strain, which is considered to belong to *Klebsiella pneumoniae,* from the intestines of the mice in which this increase in Th1 cells was observed. Furthermore, it was also clarified that the bacteria derived from the saliva of patients with Crohn's disease were involved in the development of enteritis by colonizing in the intestinal tract and inducing the proliferation or activation of Th1 cells.
[0007] Moreover, the present inventors found that when Kp2H7 strains were orally administered to SPF (specific-pathogen-free) mice, intestinal colonization of these bacterial strains was not

observed, unlike the case of the germ-free mice. Furthermore, it was also clarified that by administering an antibiotic to the SPF mice, these bacterial strains might be able to colonize in the intestinal tracts of the mice. Then, from these results, the present inventors considered that the intestinal tract contains intestinal bacteria that inhibit the intestinal colonization of Th1 cell-inducible bacteria such as the Kp2H7 strain, and administration of the antibiotic eliminates the intestinal bacteria from the intestinal tract, making the intestinal colonization of the bacteria possible.

[0008] Therefore, the present inventors tried to identify the bacteria that suppress the intestinal colonization of Th1 cell-inducible bacteria among human intestinal bacteria. As a result, the present inventors succeeded in isolating and culturing 68 strains, 37 strains, and 42 strains of intestinal bacterial strains from fecal samples derived from 3 healthy subjects (#K, #F, and #I), respectively, and in determining the sequence of 16S rDNA of each bacterial strain. Furthermore, it was clarified that administration of these bacterial strains suppressed intestinal colonization of Th1 cell-inducible bacteria (PTL 2).

CITATION LIST

Patent Literature

[0009] [PTL 1] International Publication No. WO2018/084172 [0010] [PTL 2] International Publication No. WO2019/017389

Non Patent Literature

[0011] [NPL 1] Y. Chen et al., Scientific reports 6, 34055 (2016) [0012] [NPL 2] D. Gevers et al., Cell host & microbe 15, 382-392 (2014) [0013] [NPL 3] C. A. Lozupone et al., Cell host & microbe 14, 329-339 (2013) [0014] [NPL 4] I. Vujkovic-Cvijin et al., Science translational medicine 5, 193ra191 (2013) [0015] [NPL 5] N. Qin et al., Nature 513, 59-64 (2014) [0016] [NPL 6] C. L. Sears, W. S. Garrett, Cell host & microbe 15, 317-328 (2014)

SUMMARY OF INVENTION

Technical Problem

[0017] The present invention has an object to find an intestinal bacterium having antibacterial activity against drug-resistant bacteria or pro-inflammatory bacteria, and to provide an antibacterial composition against drug-resistant bacteria or pro-inflammatory bacteria containing the intestinal bacterium as an active ingredient as well as a pharmaceutical composition or method for treating, ameliorating, or preventing diseases caused by drug-resistant bacteria or pro-inflammatory bacteria.

Solution to Problem

[0018] The present inventors have made earnest studies to achieve the above object, and have clarified as a result that the above-mentioned bacteria that suppress the intestinal colonization of Th1 cell-inducible bacteria (68 strains of intestinal bacteria derived from healthy subject #K, 37 strains of intestinal bacteria derived from healthy subject #F, and 42 strains of intestinal bacteria derived from healthy subject #I) can suppress the intestinal colonization of multidrug-resistant bacteria (carbapenem-resistant Enterobacteriaceae, vancomycin-resistant enterococci, *Clostridium difficile, Campylobacter jejuni*) and pro-inflammatory bacteria (adherent-invasive *Escherichia coli*).

[0019] Furthermore, regarding the ability to suppress bacterial colonization in the intestine, the present inventors have succeeded in selecting 18 strains from the 37 strains of intestinal bacteria derived from healthy subject #F, which are capable of exerting the same level of suppression ability as the 37 strains. Thus, the present invention has been completed.

[0020] Specifically, the present invention provides the following.

[0021] [1] An antibacterial composition against drug-resistant bacteria or pro-inflammatory bacteria comprising: an intestinal bacterium as an active ingredient.

[0022] [2] The antibacterial composition according to [1], wherein the intestinal bacterium is at least one bacterium which includes a DNA composed of a base sequence set forth in any of SEQ ID NOS: 69 to 105 or a base sequence having at least 90% identity to the base sequence.

[0023] [3] The antibacterial composition according to [1], wherein the intestinal bacterium is at least one bacterium which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 69, 80, 85 to 92, 94, 96, 98 to 101, 103, and 105 or a base sequence having at least 90% identity to the base sequence.

[0024] [4] The antibacterial composition according to [1], wherein the intestinal bacterium is at least one bacterium specified by any of accession numbers NITE BP-03147 to 03164.

[0025] [5] The antibacterial composition according to [1], wherein the intestinal bacterium is at least one bacterium which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 1 to 147 or a base sequence having at least 90% identity to the base sequence.

[0026] [6] The antibacterial composition according to [1], wherein the intestinal bacterium is at least one bacterium which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 1 to 68 or a base sequence having at least 90% identity to the base sequence.

[0027] [7] The antibacterial composition according to [1], wherein the intestinal bacterium is at least one bacterium which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 106 to 147 or a base sequence having at least 90% identity to the base sequence.

[0028] [8] The antibacterial composition according to any one of [1] to [7], which is a pharmaceutical composition.

[0029] [9] The antibacterial composition according to any one of [1] to [7], which is a pharmaceutical composition for treating, ameliorating, or preventing an infectious disease or an inflammatory disease.

Advantageous Effects of Invention

[0030] According to the present invention, by suppressing the colonization and the like of drug-resistant bacteria or pro-inflammatory bacteria in the intestinal tract, it is possible to suppress the proliferation or activation of these bacteria, and it is possible to treat, ameliorate, or prevent diseases caused by these bacteria.

Description

BRIEF DESCRIPTION OF DRAWINGS

[0031] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0032] FIG. **1** is a graph in CFU showing changes over time in the bacterial amount of fecal *Klebsiella* when *Klebsiella* 2H7 strains (Kp2H7) were administered to germ-free mice, and one week later, fecal samples of healthy subjects were administered to the mice (FMT). Five types of feces were used, and *Klebsiella* was significantly reduced in all samples.

[0033] FIG. **2** is a bar graph showing the results of 16S meta-analysis of three types of feces derived from healthy donors F, I, and K. Each segment indicates one bacterial strain, and its size indicates the proportion of that bacterium to the total bacterial amount. The three types of feces were cultured in an anaerobic environment, and the strains that could be cultured and isolated there are shown in yellow in the adjacent graph (under color display). The total number of bacteria that could be isolated is shown below.

[0034] FIG. **3** is a graph in CFU showing changes over time in the bacterial amount of fecal Kp2H7 when Kp2H7 was colonized in germ-free mice and then the bacterial strains isolated from feces were mixed and administered. The 37 strains derived from the feces derived from healthy donor F (feces F) reduced *Klebsiella* as much as the fecal sample.

[0035] FIG. **4** is a graph in CFU showing changes over time in the bacterial amount of fecal Kp2H7 when Kp2H7 was colonized in germ-free mice and then the bacterial strains isolated from feces were mixed and administered. The 37 strains derived from feces F and 68 bacterial strains

isolated from the feces derived from healthy donor K (feces K) reduced *Klebsiella* as much as the fecal sample.

[0036] FIG. **5** is a graph in CFU showing changes over time in the bacterial amount of fecal Kp2H7 when Kp2H7 was administered to germ-free mice, then F37mix (37 bacterial strains derived from feces F) was administered one week later, and ampicillin was further administered by drinking water one month later. *Klebsiella* transiently increased with ampicillin administration, but then decreased again.

[0037] FIGS. **6**A**1**-**6**A**4** are diagrams showing changes over time in the abundance ratio of each bacterial amount (F31, F22, F20, F32) in the total bacterial amount in the experiment shown in FIG. **5**. Below the figure, the number of each bacterium, r (Spearman's rank correlation coefficient with *Klebsiella*), and the bacterium's name are shown.

[0038] FIGS. **6**B**1**-**6**B**4** are diagrams showing changes over time in the abundance ratio of each bacterial amount (F26, F28, F21, F30) in the total bacterial amount in the experiment shown in FIG. **5**. Below the figure, the number of each bacterium, r (Spearman's rank correlation coefficient with *Klebsiella*), and the bacterium's name are shown.

[0039] FIGS. **6**C**1**-**6**C**4** are diagrams showing changes over time in the abundance ratio of each bacterial amount (F24, F23/F25, F35/F36, F09) in the total bacterial amount in the experiment shown in FIG. **5**. Below the figure, the number of each bacterium, r (Spearman's rank correlation coefficient with *Klebsiella*), and the bacterium's name are shown.

[0040] FIGS. **6**D**1**-**6**D**4** are diagrams showing changes over time in the abundance ratio of each bacterial amount (F33, F12, F17/F19, F18) in the total bacterial amount in the experiment shown in FIG. **5**. Below the figure, the number of each bacterium, r (Spearman's rank correlation coefficient with *Klebsiella*), and the bacterium's name are shown.

[0041] FIGS. **6**E**1**-**6**E**4** are diagrams showing changes over time in the abundance ratio of each bacterial amount (F34, F03/F08, F29, F13) in the total bacterial amount in the experiment shown in FIG. **5**. Below the figure, the number of each bacterium, r (Spearman's rank correlation coefficient with *Klebsiella*), and the bacterium's name are shown.

[0042] FIGS. **6**F**1**-**6**F**4** are diagrams showing changes over time in the abundance ratio of each bacterial amount (F04/F08, F37, F01, F02) in the total bacterial amount in the experiment shown in FIG. **5**. Below the figure, the number of each bacterium, r (Spearman's rank correlation coefficient with *Klebsiella*), and the bacterium's name are shown.

[0043] FIGS. **6**G**1**-**6**G**3** are diagrams showing changes over time in the abundance ratio of each bacterial amount (F05, F07, F14) in the total bacterial amount in the experiment shown in FIG. **5**. Below the figure, the number of each bacterium, r (Spearman's rank correlation coefficient with *Klebsiella*), and the bacterium's name are shown.

[0044] FIGS. **6**H**1**-**6**H**3** are diagrams showing changes over time in the abundance ratio of each bacterial amount (F10/F15, F16, F11/F27) in the total bacterial amount in the experiment shown in FIG. **5**. Below the figure, the number of each bacterium, r (Spearman's rank correlation coefficient with *Klebsiella*), and the bacterium's name are shown.

[0045] FIG. **7** is a diagram in which bacteria are arranged in the order of positive correlation in Spearman's rank correlation coefficient between Kp2H7 and each bacterium in terms of bacterial amount. Many of the *Bacteroides* are not related in movement to Kp2H7, and many of the negatively correlated ones are in the *Firmicutes* genus.

[0046] FIG. **8** is a graph in CFU showing changes over time in the bacterial amount of fecal Kp2H7 when Kp2H7 was administered to germ-free mice for colonization, and then the 37 strains shown in FIG. **7** (F37mix), 8 strains of the 37 strains belonging to the Bacteroidetes genus (F8mix), or the 29 other bacterial strains (F29mix) were mixed and administered. The 29 strains excluding *Bacteroides* also showed a decrease in *Klebsiella* that was almost the same as the 37 strains, and it is considered that *Bacteroides* is unnecessary for the elimination of *Klebsiella*.

[0047] FIG. **9** is a phylogenetic tree showing a breakdown of F37mix, F8mix, and F29mix used in

the experiment shown in FIG. **8**. The phylogenetic tree was prepared by the Neighbor-joining method using MEGA X with the DNA base sequence of the 16S rDNA analysis result of the isolated bacteria by the Sanger method. The same applies to the preparation of the phylogenetic trees in FIGS. **10** and **12**.

[0048] FIG. **10** is a phylogenetic tree showing a breakdown of 18 bacterial strains derived from feces F (F18mix).

[0049] FIG. **11** is a graph in CFU showing changes over time in the bacterial amount of fecal Kp2H7 when Kp2H7 was colonized in germ-free mice, and then 37 bacterial strains derived from feces F (F37mix described above), 18 bacterial strains derived from feces F (F18mix shown in FIG. **10**), or 42 bacterial strains derived from the feces derived from healthy subject I (feces I) were mixed and administered. F18mix also successfully eliminated *Klebsiella* in the same way as F37mix.

[0050] FIG. **12** is a phylogenetic tree that classifies 18 bacterial strains derived from feces F (F18mix) into 4 groups and shows the breakdown thereof. These 4 groups were subtracted from the 18 bacterial strains (F18mix) to prepare bacterial strain groups of F15mix (F18mix-other phyla), F12mix (F18mix-Lachnoclostridium), F14mix (F18mix-Blautia), and F13mix (F18mix-other *Firmicutes*), which were subjected to the experiment shown in FIG. **11**.

[0051] FIG. **13** is a graph in CFU showing changes over time in the bacterial amount of fecal Kp2H7 when Kp2H7 was colonized in germ-free mice, and then the group excluding the duplicate strains and F18mix from F37mix (F31-18mix), F15mix described above, F12mix described above, F14mix described above, F13mix described above, or F18mix described above was mixed and administered. Note that FIG. **13** shows data from the experiments performed twice. Excluding any group shown in FIG. **12** from F18mix decreased the ability to eliminate *Klebsiella*, and it has become clear that every group is important for the elimination of *Klebsiella*.

[0052] FIG. **14** is a graph showing the CFU of fecal Kp2H7 in each group at the time of day 28 in the experiment shown in FIG. **13**. The F18mix-administered group has a statistically significantly smaller bacterial amount of *Klebsiella* than the other administration groups excluding F37mix.

[0053] FIG. **15** is a dot plot diagram showing the results of flow cytometric analysis of immune cells in the lamina propria of the large intestine of mice in the F37mix-administered group, the F18mix-administered group, or the Kp2H7 alone administration group. The numerical value in each gate (square) in the figure shows the proportion of CD4+IFNγ+ cells. The induction of CD4+IFNγ+ cells is suppressed in the F37mix-administered group and the F18mix-administered group as compared with the Kp2H7 alone administration group.

[0054] FIG. **16** is a graph in CFU showing changes over time in the bacterial amount of fecal Kp2H7 when Kp2H7 was administered to germ-free mice, and one week later, each bacterial strain mix was administered. The "F15mix" in the figure shows the result of mice administered by removing three strains of *E. coli, Fusobacterium,* and *Bifidobacterium* from F18mix, and "F18mix-*E. coli*", "F18mix-*Fusobacterium*," and "F18mix-*Bifidobacterium*" each show the result of mice administered by removing one of the three strains from F18mix. The effects of all three strains diminished when removed from F18mix, indicating that each was involved in the elimination of *Klebsiella.*

[0055] FIG. **17** is a graph in CFU showing changes over time in the bacterial amount of fecal Kp2H7 when Kp2H7 was administered to a germ-free Rag2.sup.−/−γc.sup.−/− mouse, MyD88.sup.−/−Triff.sup.−/− mouse, or wild-type mouse (WT), and one week later, mixed F37mix was administered. *Klebsiella* was equally eliminated in all types of mice. This suggests that the major innate immunity and acquired immunity of the host are not involved in the elimination of *Klebsiella.*

[0056] FIG. **18** is a graph in CFU showing changes over time in the bacterial amount of fecal CRE when *Klebsiella* (Kp-CRE) was administered to germ-free mice, and one week later, the isolated bacteria mixes (F37mix, K68mix, I42mix) were administered to the mice. F37mix and K68mix can

also reduce CRE.

[0057] FIG. **19** is a photomicrograph showing the results of analyzing the large intestine of a mouse at the end of the experiment shown in FIG. **18** by HE staining. No inflammatory findings were observed in any of the isolated bacterium mix-administered mice.

[0058] FIG. **20** is a graph in CFU showing changes over time in the bacterial amount of fecal VRE when VRE (vancomycin-resistant enterococci) was administered to germ-free mice, and one week later, the isolated bacteria mixes (F37mix, K68mix, I42mix) were administered to the mice. For VRE, K68mix was able to reduce the bacterial amount more than F37mix.

[0059] FIG. **21** is a photomicrograph showing the results of analyzing the large intestine of a mouse at the end of the experiment shown in FIG. **18** by HE staining. No inflammatory findings were observed in any of the isolated bacterium mix-administered mice.

[0060] FIG. **22** is a graph in CFU showing changes over time in the bacterial amount of fecal AIEC when AIEC was administered to germ-free mice, and one week later, the isolated bacteria mixes (F37mix, K68mix, I42mix) were administered to the mice. For AIEC, F37mix was most effective in reducing the bacterial amount.

[0061] FIG. **23** is a graph in CFU showing changes over time in the bacterial amount of fecal ESBL-producing *Klebsiella* when ESBL-producing *Klebsiella* was administered to germ-free mice, and one week later, the isolated bacterium mixes (F37mix, K68mix, I42mix) were administered to the mice. F37mix and K68mix were able to eliminate ESBL-producing *Klebsiella* as much as F-derived feces.

[0062] FIG. **24** is a graph in CFU showing changes over time in the bacterial t of fecal *Campylobacter* when *Campylobacter jejuni* was administered to germ-free mice, and one week later, fecal samples derived from the isolated bacteria mixes (F37mix, K68mix, I42mix) or healthy subject F were administered to the mice. Elimination of *Campylobacter jejuni* was observed to the same extent in all the isolated bacteria mix-administered groups.

[0063] FIG. **25** is a graph showing changes over time in the bacterial amount of fecal *Campylobacter* as a relative value divided by the total bacterial amount when *Campylobacter jejuni* was administered to germ-free mice, and one week later, fecal samples derived from the isolated bacteria mixes (F37mix, K68mix, I42mix) or healthy subject F were administered to the mice. Elimination of *Campylobacter jejuni* was observed to the same extent in all the isolated bacteria mix-administered groups.

[0064] FIG. **26** is a graph showing the results of qPCR analysis of changes over time in the bacterial amount of fecal *Clostridium difficile* when *Clostridium difficile* was administered to germ-free mice, and one week later, fecal samples derived from the isolated bacteria mixes (F37mix, K68mix, I42mix, K47mix) or healthy subject F were administered to the mice. K68mix and K47mix were able to eliminate *Clostridium difficile* as compared with F-derived feces, but the elimination effect of F37mix was lower.

BIOLOGICAL MATERIAL DEPOSIT INFORMATION

[0065] Applicants made the following biological deposits under the terms of the Budapest Treaty on the International Recogni tion of the Deposit of Microorganisms for the Purposes of Pate nt Procedure at NITE Patent Microorganisms Depositary, Nation al Institute of Technology and Evalution, #122, 2-5-8 Kazusak amatari, Kisarazu-shi, Chiba 292-0818, Japan.:

TABLE-US-00001 Depositor International Identification Depository Reference Designation Date of Deposit *Klebsiella pneumonia* NITE BP-04150 Sep. 3, 2024 Kp-2H7 strain *Klebsiella aeromobilis* NITE BP-04151 Sep. 3, 2024 Ka-11E12 strain

DESCRIPTION OF EMBODIMENTS

<Intestinal Bacteria>

[0066] In the present invention, the intestinal bacteria contained as an active ingredient of the antibacterial composition have an antibacterial action against drug-resistant bacteria or pro-inflammatory bacteria (hereinafter also referred to as "drug-resistant bacteria and the like") in the

intestinal tract.

[0067] In the present invention, the "antibacterial activity" means an activity of suppressing the activity of bacteria, more specifically, an activity of suppressing the proliferation or colonization of bacteria or killing bacteria, and examples thereof include the activity of suppressing the colonization of bacteria in the intestines and the activity of eliminating bacteria from the intestines.

[0068] The "Intestinal bacteria" mean bacteria present in the intestinal tract of an animal. Examples of animals in which such bacteria are present include humans and non-human animals (such as mice, rats, monkeys, pigs, cattle, horses, sheep, goats, chickens, ducks, ostriches, domestic ducks, dogs, cats, rabbits, and hamsters), but among these animals, humans are preferable.

[0069] In the present invention, the "intestinal bacteria" may be a single strain of bacteria or a mixture of bacterial strains composed of multiple strains of bacteria. Note that when composed of multiple strains of bacteria, it is desirable that at least one of the bacterial strains has antibacterial activity against drug-resistant bacteria and the like. Further, in such a case, the multiple strains of bacteria may even include a bacterial strain that does not have the antibacterial activity, such as a bacterial strain having an action of enhancing the activity of a bacterial strain having the antibacterial activity, a bacterial strain having an action of maintaining the proliferation or colonization of a bacterial strain having antibacterial activity, and a bacterial strain having an action of suppressing the inhibitory activity of a bacterium that inhibits the antibacterial activity.

[0070] In the present invention, examples of the "intestinal bacteria" include at least one bacterium having a DNA composed of a base sequence set forth in any of SEQ ID NOs: 1 to 147 or a base sequence having at least 70% identity to the base sequence, at least one bacterium having a DNA composed of a base sequence set forth in any of SEQ ID NOs: 1 to 68 or a base sequence having at least 70% identity to the base sequence, at least one bacterium having a DNA composed of a base sequence set forth in any of SEQ ID NOs: 69 to 105 or a base sequence having at least 70% identity to the base sequence (for example, at least one bacterium having a DNA composed of a base sequence set forth in any of SEQ ID NOS: 69, 80, 85 to 92, 94, 96, 98 to 101, 103, and 105 or a base sequence having at least 70% identity to the base sequence), and at least one bacterium having a DNA composed of a base sequence set forth in any of SEQ ID NOS: 106 to 147 or a base sequence having at least 70% identity to the base sequence.

[0071] The sequence indicated by each SEQ ID NO is the sequence of 16S rDNA of each bacterium K68, F37, and 143 in the attached document. Tables 1 to 4 below show the correspondence between each bacterium, the SEQ ID NO indicating the sequence of each 16S rDNA, and each bacterial species estimated from the sequence. In addition, K, F, and I represent intestinal bacteria isolated from the feces of three healthy subjects (Japanese) (see PTL 2).

TABLE-US-00002 TABLE 1 SEQ ID NCBI % No. Species NO: subject id TAX ID identity K01 *Drancourtella* 1 NR_144722.1 1632013 99.57 *massiliensis* K02 *Bacteroides ovatus* 2 NR_112940.1 28116 99.86 K03 *Blautia coccoides* 3 NR_104700.1 1532 99.86 K04 *Blautia hominis* 4 NR_163638.1 2025493 99.43 K05 *Desulfovibrio vutgaris* 5 NR_074446.1 882 91.58 K06 *Alistipes onderdonkii* 6 NR_043318.1 328813 99.86 K07 *Eisenbergiella* 7 NR_144731.1 1720294 99.50 *massiliensis* K08 *[Clostridium]* 8 NR_029164.1 1522 97.93 *innocuum* K09 *Bacteroides fragilis* 9 NR_112936.1 817 99.71 K10 *Eggerthella lenta* 10 NR_074377.1 84112 100.00 K11 *Dielma fastidiosa* 11 NR_125593.1 1034346 99.71 K12 *Erysipelatoclostridium* 12 NR_113243.1 1547 100.00 *ramosum* K13 *Enterococcus faecalis* 13 NR_115765.1 1351 99.93 K14 *Bacteroides* 14 NR_041307.1 329854 99.35 *intestinalis* K15 *[Clostridium]* 15 NR_118730.1 1512 98.35 *symbiosum* K16 *[Clostridium]* 16 NR_024719.1 89153 99.50 *hylemonae* K17 *Hungatella effluvii* 17 NR_133762.1 1096246 98.49 K18 *Bacteroides dorei* 18 NR_041351.1 357276 99.93 K19 *[Clostridium]* 19 NR_044715.2 1531 98.99 *clostridioforme* K20 *Flavonifractor plautii* 20 NR_029356.1 292800 100.00 K21 *Bacteroides* 21 NR_112947.1 657309 99.50 *xylanisolvens* K22 *Bacteroides* 22 NR_112944.1 818 99.93 *thetaiotaomicron* K23 *Parabacteroides* 23 NR_041343.1 46503 99.78 *merdae* K24 *Bacteroides vulgatus* 24 NR_074515.1 435590 100.00 K25

[Clostridium] 25 NR_043680.1 358742 99.41 aldenense K26 Bacteroides uniformis 26 NR_112945.1 820 97.39 K27 Gordonibacter 27 NR_148261.1 1335613 99.56 urolithinfaciens K28 Coprococcus comes 28 NR_044048.1 470146 92.77 K29 Anaerostipes caccae 29 NR_028915.1 105841 98.78 K30 [Ruminococcus] 30 NR_036800.1 411470 99.78 gnavus K31 [Ruminococcus] 31 NR_036800.1 411470 98.71 gnavus K32 Alistipes shahii 32 NR_113153.1 328814 100.00 K33 Bacteroides stercoris 33 NR_112943.1 449673 98.77 K34 Blautia hominis 34 NR_163634.1 2025493 98.71

TABLE-US-00003 TABLE 2 SEQ ID NCBI % No. Species NO: subject id TAX ID identity K35 Butyricicoccus 35 NR_152060.1 1712515 97.89 faecihominis K36 [Clostridium] bolteae 36 NR_025567.1 208479 99.50 K37 Phocea massiliensis 37 NR_144748.1 1841867 99.93 K38 Holdemania 38 NR_125628.1 1211819 99.71 massiliensis K39 Escherichia coli 39 NR_114042.1 582 99.79 K40 Agathobaculum 40 NR_044644.2 39484 96.68 desmolans K41 [Eubacterium] rectale 41 NR_074634.1 515619 100.00 K42 Lactonifactor 42 NR_043551.1 341220 100.00 longoviformis K43 Oscillibacter 43 NR_118156.1 1007096 95.90 ruminantium K44 Pseudoflavonifractor 44 NR_147370.1 1870988 97.42 phocaeensis K45 Streptococcus 45 NR_043660.1 197614 100.00 pasteurianus K46 Sutterella 46 NR_117778.1 40545 99.93 wadsworthensis K47 Bifidobacterium 47 NR_133982.1 1454229 99.35 faecale K48 Eisenbergiella 48 NR_144731.1 1720294 93.50 massiliensis K49 Fusicatenibacter 49 NR_114326.1 1150298 99.78 saccharivorans K50 Robinsoniella 50 NR_041882.1 180332 93.40 peoriensis K51 Massilimicrobiota 51 NR_144738.1 1776392 99.93 timonensis K52 Blautia faecis 52 NR_109014.1 871665 99.92 K53 Blautia wexlerae 53 NR_044054.1 1121115 98.63 K54 Phascolarctobacterium 54 NR_026111.1 33025 99.72 faecium K55 Odoribacter 55 NR_113075.1 28118 99.71 splanchnicus K56 Faecalibacterium 56 NR_028961.1 853 97.29 prausnitzii K57 Colidextribacter 57 NR_147375.1 1870986 94.70 massiliensis K58 Emergencia 58 NR_144737.1 1776384 91.61 timoriensis K59 Alistipes finegoidii 59 NR_043064.1 214856 99.42 K60 Ruthenibacterium 60 NR_151900.1 1550024 99.93 lactatiformans K61 Christensenella 61 NR_112900.1 626937 98.71 minuta K62 [Clostridium] scindens 62 NR_028785.1 29347 98.49 K63 Enterococcus faecalis 63 NR_115765.1 1351 100.00 K64 Blautia coccoides 64 NR_104700.1 1532 99.93 K65 Alistipes ihumii 65 NR_144706.1 1211813 99.28 K66 Intestinimonas 66 NR_118554.1 1297617 99.86 butyriciproducens K67 Bacteroides 67 NR_112945.1 820 99.93 uniformis K68 Akkermansia 68 NR_074436.1 239935 98.91 muciniphila

TABLE-US-00004 TABLE 3 SEQ ID NCBI % No. Species NO: subject id TAX ID identity F01 Bifidobacterium 69 NR_145535.1 1931217 99.60 longum F02 Bacteroides 70 NR_112947.1 657309 99.59 xylanisolvens F03 Bacteroides fragilis 71 NR_074784.2 817 98.95 F04 Bacteroides uniformis 72 NR_112945.1 820 99.93 F05 Bacteroides 73 NR_074277.1 818 99.80 thetaiotaomicron F06 Bacteroides uniformis 74 NR_112945.1 820 99.93 F07 Bacteroides 75 NR_112931.1 85831 97.18 acidifaciens F08 Bacteroides fragilis 76 NR_074784.2 817 98.95 F09 Parabacteroides 77 NR_113076.1 927665 98.98 goldstenii F10 [Ruminococcus] 78 NR_036800.1 411470 99.80 gnavus F11 Blautia luti 79 NR_114315.l 649762 97.57 F12 Faecalimonas 80 NR_156907.1 1912855 99.87 umbilicata F13 [Clostridium] 81 NR_102852.1 84030 94.34 saccharolyticum F14 Tyzzerella nexilis 82 NR_029248.1 500632 98.49 F15 [Ruminococcus] 83 NR_036800.1 411470 99.80 gnavus F16 Anaerostipes hadrus 84 NR_117138.2 649756 99.74 F17 Blautia glucerasea 85 NR_113231.1 536633 96.91 F18 [Clostridium] bolteae 86 NR_025567.1 208479 99.60 F19 Blautia caecimuris 87 NR_144607.1 1796615 99.13 F20 [Clostridium] 88 NR_029164.1 1522 98.71 innocuum F21 Blautia marasmi 89 NR_147395.1 1917868 98.20 F22 [Clostridium] 90 NR_044289.1 460384 98.97 lavalense F23 [Clostridium] 91 NR_112553.1 342942 99.13 glycyrrhizinilyticum F24 Eisenbergiella 92 NR_144731.1 1720294 97.73 massiliensis F25 [Clostridium] 93 NR_112553.1 342942 99.13 glycyrrhizinilyticum F26 Flavonifractor plautii 94 NR_029356.1 292800 100.00 F27 Blautia luti 95 NR_114315.1 649762 97.57 F28 Intestinibacter bartiettii 96 NR_027573.1 261299 99.93 F29 [Ruminococcus] 97

NR_036800.1 411470 98.85 *gnavus* F30 *Massilimicrobiota* 98 NR_144738.1 1776392 100.00 *timonensis* F31 *Anaerostipes caccae* 99 NR_028915.1 105841 97.32 F32 *Blautia coccoides* 100 NR_104700.1 1532 99.93 F33 *Eysipelatoclostridium* 101 NR_029247.1 1547 99.41 *ramosum* F34 *Veillonella parvula* 102 NR_074980.1 479436 99.16 F35 *Fusobacterium varium* 103 NR_113384.1 856 99.73 F36 *Fusobacterium varium* 104 NR_113384.1 856 99.73 F37 *Escherichia fergusonii* 105 NR_074902.1 585054 99.87

TABLE-US-00005 TABLE 4 SEQ ID NCBI % No. Species NO: subject id TAX ID identity I01 *Bifidobacterium* 106 NR_133982.1 1454229 98.06 *faecale* I02 *Bifidobacterium* 107 NR_037117.1 28026 99.63 *pseudocatenulatum* I03 *Bifidobacterium* 108 NR_044771.1 1681 100.00 *bifidum* I04 *Bifidobacterium* 100 NR_145535.1 1931217 99.70 *longum* I05 *Collinsella aerofaciens* 110 NR_113316.1 74426 99.92 I06 *Collinsella aerofaciens* 111 NR_113316.1 74426 99.72 I07 *Bifidobacterium* 112 NR_145535.1 1931217 99.70 *longum* I08 *Bacteroides stercoris* 113 NR_112943.1 449673 99.18 I09 *Bacteroides* 114 NR_042745.1 204516 99.70 *massiliensis* I10 *Bacteroides vulgatus* 115 NR_074515.1 435590 100.00 I11 *Bacteroides dorei* 116 NR_041351.1 357276 100.00 I12 *Parabacteroides* 117 NR_041343.1 46503 99.85 *merdae* I13 *Parabacteroides* 118 NR_041342.1 823 99.03 *distasonis* I14 *Alistipes putredinis* 119 NR_113152.1 28117 100.00 I15 *Bacteroides uniformis* 120 NR_040866.1 820 97.75 I16 *Bacteroides koreensis* 121 NR_159117.1 1912896 99.26 I17 *Alistipes shahii* 122 NR_113153.1 328814 99.63 I18 *Odoribacter* 123 NR_113075.1 28118 99.77 *splanchnicus* I19 *Faecalibacterium* 124 NR_028961.1 853 97.24 *prausnitzii* I20 *Faecalibacterium* 125 NR_028961.1 853 97.24 *prausnitzii* I21 *Blautia wexlerae* 126 NR_044054.1 1121115 98.38 I22 *Ruminococcus lactaris* 127 NR_027579.1 471875 97.24 I23 *Ruminococcus albus* 128 NR_113032.1 1264 94.68 I24 *Faecalibacterium* 129 NR_028961.1 853 96.86 *prausnitzii* I25 *Dorea longicatena* 130 NR_028883.1 88431 99.47 I26 *Dorea formicigenerans* 131 NR_044645.2 39486 97.94 I27 *Anaerostipes hadrus* 132 NR_117138.2 649756 99.85 I28 *Intestinibacter* 133 NR_027573.1 261299 99.55 *bartlettii* I29 *Flavonifractor plautii* 134 NR_029356.1 292800 99.71 I30 *Pseudoflavonifractor* 135 NR_147370.1 1870988 97.86 *phocaeensis* I31 *[Clostridium]* 136 NR_114393.1 29348 93.09 *spiroforme* I32 *Megasphaera elsdenii* 137 NR_102980.1 1064535 99.35 I33 *Dialister* 138 NR_041666.1 742743 97.02 *succinatiphilus* I34 *Acidaminococcus* 139 NR_041894.1 187327 99.93 *intestini* I35 *Allisonella* 140 NR_028862.1 209880 99.50 *histaminiformans* I36 *Megasphaera* 141 NR_133027.1 1232428 99.00 *massiliensis* I37 *Sutterella* 142 NR_104851.1 40545 99.78 *wadsworthensis* I38 *Clostridium baratii* 143 NR_029229.1 1561 99.85 I39 *Anaeromassilibacillus* 144 NR_144727.1 1673717 97.27 *senegalensis* I40 *Colidextribacter* 145 NR_147375.1 1870986 95.00 *massiliensis* I41 *Flavonifractor plautii* 146 NR_029356.1 292800 97.96 I42 *[Clostridium] leptum* 147 NR_114789.1 1535 94.55

[0072] Tables 1 to 4 show the top-hit species names and RefSeq accessions as a result of a BLAST search for the sequences set forth in the SEQ ID NOs on the RefSeq 16s DNA sequence database (as of Jan. 8, 2020). Note that it is generally said % identity>97% can identify species, and >94% can identify genus. Therefore, it should be understood that bacterial strains with a % identity of >94% are bacteria that can be identified at the genus level.

[0073] The "at least 70% identity" in the intestinal bacteria of the present invention means that the identity to each base sequence is preferably 80% or more, more preferably 85% or more, further preferably 90% or more (for example, 91% or more, 92% or more, 93% or more, and 94% or more), more preferably 94% or more (for example, 95% or more, 96% or more, 97% or more, and 98% or more), and particularly preferably 99% or more.

[0074] In addition, the homology or identity of a sequence (amino acid sequence or nucleotide (base) sequence) can be determined by using the BLAST (Basic Local Alignment Search) program (Altschul et al. J. Mol. Biol., 215:403-410, 1990). The program is based on the algorithm BLAST by Karlin and Altschul (Proc. Natl. Acad. Sci. USA, 87:2264-2268, 1990, Proc. Natl. Acad. Sci. USA, 90:5873-5877, 1993). When analyzing the homology or identity between sequences by

BLAST, it can be determined by using, for example, the BLAST of the National Center for Biotechnology Information (NCBI) (for example, by using the default, that is, initially set parameters).

[0075] In the present invention, the "intestinal bacterium" which includes a DNA composed of a base sequence set forth in any of SEQ ID NOS: 1 to 147 or a base sequence having at least 70% identity to the base sequence is preferably at least 15 bacteria in the intestinal bacterial group, more preferably at least 30 bacteria in the intestinal bacterial group, further preferably at least 75 bacteria in the intestinal bacterial group, more preferably at least 120 bacteria in the intestinal bacterial group, further preferably at least 135 bacteria in the intestinal bacterial group, more preferably at least 140 bacteria in the intestinal bacterial group, further preferably 147 intestinal bacteria each of which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 1 to 147 or a base sequence having at least 70% identity to the base sequence, and particularly preferably 147 bacteria each of which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 1 to 147.

[0076] In the present invention, the "intestinal bacterium" which includes a DNA composed of a base sequence set forth in any of SEQ ID NOS: 1 to 68 or a base sequence having at least 70% identity to the base sequence is preferably at least 7 bacteria in the intestinal bacterial group, more preferably at least 15 bacteria in the intestinal bacterial group, further preferably at least 35 bacteria in the intestinal bacterial group, more preferably at least 60 bacteria in the intestinal bacterial group, further preferably at least 65 bacteria in the intestinal bacterial group, more preferably 68 intestinal bacteria each of which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 1 to 68 or a base sequence having at least 70% identity to the base sequence, and particularly preferably 68 bacteria each of which includes a DNA composed of a base sequence set forth in any of SEQ ID NOS: 1 to 68. In addition, the "intestinal bacterium" which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 1 to 68 or a base sequence having at least 70% identity to the base sequence desirably has resistance to ampicillin. Also, the present invention uses 46 bacteria each of which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 1 to 46 or a base sequence having at least 70% identity to the base sequence.

[0077] In the present invention, the "intestinal bacterium" which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 69 to 105 or a base sequence having at least 70% identity to the base sequence is preferably at least 4 bacteria in the intestinal bacterial group, more preferably at least 8 bacteria in the intestinal bacterial group, further preferably at least 18 bacteria in the intestinal bacterial group, more preferably at least 29 bacteria in the intestinal bacterial group, further preferably at least 33 bacteria in the intestinal bacterial group, more preferably at least 35 bacteria in the intestinal bacterial group, further preferably 37 intestinal bacteria each of which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 69 to 105 or a base sequence having at least 70% identity to the base sequence, and particularly preferably 37 bacteria each of which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 69 to 105. In addition, the "intestinal bacterium" which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 69 to 105 or a base sequence having at least 70% identity to the base sequence desirably has sensitivity to ampicillin.

[0078] In the present invention, the "intestinal bacterium" which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 69, 80, 85 to 92, 94, 96, 98 to 101, 103, and 105 or a base sequence having at least 70% identity to the base sequence is preferably at least 2 bacteria in the intestinal bacterial group, more preferably at least 5 bacteria in the intestinal bacterial group, further preferably at least 10 bacteria in the intestinal bacterial group, more preferably at least 14 bacteria in the intestinal bacterial group, further preferably at least 15 bacteria in the intestinal bacterial group, more preferably at least 16 bacteria in the intestinal bacterial group, further preferably at least 17 bacteria in the intestinal bacterial group, more preferably 18 intestinal bacteria each of which includes a DNA composed of a base sequence set forth in any of SEQ ID

NOs: 69, 80, 85 to 92, 94, 96, 98 to 101, 103, and 105 or a base sequence having at least 70% identity to the base sequence, and particularly preferably 18 bacteria each of which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 69, 80, 85 to 92, 94, 96, 98 to 101, 103, and 105 (bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 69, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 80, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 85, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 86, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 87, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 88, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 89, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 90, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 91, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 92, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 94, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 96, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 98, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 99, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 100, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 101, bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 103, and bacteria which include a DNA composed of a base sequence set forth in SEQ ID NO: 105).

[0079] Note that typical examples of 18 bacteria each of which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 69, 80, 85 to 92, 94, 96, 98 to 101, 103, and 105 are accession bacterial strains shown in Table 5 below. All of the bacterial strains were deposited with the National Institute of Technology and Evaluation (NITE, 2-5-8 Kazusakamatari, Kisarazu City, Chiba Prefecture, Room 122) on Mar. 2, 2020.

TABLE-US-00006 TABLE 5

Number	16S rDNA (SEQ ID NO:)	Identification Number	Accession Receipt Number	Accession Number Indication
F01	69	f01_42H6	NITE ABP-03147	NITE BP-03147
F12	80	f12_42H4	NITE ABP-03148	NITE BP-03148
F17	85	f17_42I7	NITE ABP-03149	NITE BP-03149
F18	86	f18_42I2	NITE ABP-03150	NITE BP-03150
F19	87	f19_43G2	NITE ABP-03151	NITE BP-03151
F20	88	f20_43G1	NITE ABP-03152	NITE BP-03152
F21	89	f21_42A8	NITE ABP-03153	NITE BP-03153
F22	90	f22 _43C3	NITE ABP-03154	NITE BP-03154
F23	91	f23_42K4	NITE ABP-03155	NITE BP-03155
F24	92	f24_42I4	NITE ABP-03156	NITE BP-03156
F26	94	f26_42K2	NITE ABP-03157	NITE BP-03157
F28	96	f28_43A3	NITE ABP-03158	NITE BP-03158
F30	98	f30_43A5	NITE ABP-03159	NITE BP-03159
F31	99	f31_43J5	NITE ABP-03160	NITE BP-03160
F32	100	f32_42A7	NITE ABP-03161	NITE BP-03161
F33	101	f33_43N2	NITE ABP-03162	NITE BP-03162
F35	103	f35_42L8	NITE ABP-03163	NITE BP-03163
F37	105	f37_42G1	NITE ABP-03164	NITE BP-03164

which includes a DNA composed of a base sequence set forth in any of SEQ ID NOS: 106 to 147. In addition, the "intestinal bacterium" which includes a DNA composed of a base sequence set forth in any of SEQ ID NOS: 106 to 147 or a base sequence having at least 70% identity to the base sequence desirably has sensitivity to ampicillin.

[0080] Further, in the present invention, an aspect of the "intestinal bacterium" is an intestinal bacterium that exhibits resistance to at least one compound selected from the group consisting of spectinomycin, and/or sensitivity to at least one compound selected from the group consisting of ampicillin, tylosin, and chloroform. In addition, another aspect includes intestinal bacteria that exhibit resistance to metronidazole and/or sensitivity to at least one compound selected from the group consisting of vancomycin and tylosin.

[0081] In addition, as shown in Examples described later, the above-mentioned intestinal bacteria have been isolated by the present inventors and are useful because they exert an antibacterial action against drug-resistant bacteria, pro-inflammatory bacteria, and the like. Therefore, the present

invention may also provide the following.

[0082] (1) At least one bacterium which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 69 to 105 or a base sequence having at least 90% identity to the base sequence.

[0083] (2) At least one bacterium which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 69, 80, 85 to 92, 94, 96, 98 to 101, 103, and 105 or a base sequence having at least 90% identity to the base sequence.

[0084] (3) At least one bacterium specified by any of accession numbers NITE BP-03147 to 03164.

[0085] (4) At least one bacterium which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 1 to 147 or a base sequence having at least 90% identity to the base sequence.

[0086] (5) At least one bacterium which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 69 to 105 or a base sequence having at least 90% identity to the base sequence.

[0087] (6) At least one bacterium which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 106 to 147 or a base sequence having at least 90% identity to the base sequence.

[0088] (7) The bacterium according to any one of (1) to (6), which is a bacterium having an antibacterial action against drug-resistant bacteria, pro-inflammatory bacteria, or bacteria that induce proliferation or activation of Th1 cells in the intestinal tract.

<Antibacterial Composition and Pharmaceutical Composition>

[0089] The composition of the present invention may be any as long as it contains the above-mentioned intestinal bacterium, and the bacterium may be a live bacterium or a dead bacterium. In addition, when the compositions can be used in combination, and they are ingested or absorbed in combination as a result (in the case of combined compositions), the above-mentioned intestinal bacteria can also be present separately in two or more compositions.

[0090] The composition of the present invention can be in the form of a pharmaceutical composition, a quasi-drug composition, food and drink (including animal feed), or a reagent used for research purposes (such as in vitro or in vivo experiments).

[0091] Since the composition of the present invention exhibits antibacterial activity against drug-resistant bacteria and the like, it is preferably used as a pharmaceutical composition, a quasi-drug composition, or food or drink for treating, preventing, or ameliorating diseases caused by the bacterium.

[0092] The composition of the present invention can be formulated by a known pharmaceutical method. For example, it can be used in the form of capsules, tablets, pills, liquids, powders, granules, fine granules, film coating preparations, pellets, troches, sublingual preparations, chews, buccal preparations, pastes, syrups, suspensions, elixirs, emulsions, coating preparations, ointments, platers, poultices, transdermal preparations, lotions, inhalants, aerosols, injections, suppositories, and the like, for the purpose of administration by oral, parenteral (for example, intestinal, intramuscular, intravenous, tracheal, intranasal, transdermal, intradermal, subcutaneous, intraocular, vaginal, intraperitoneal, rectal, or inhalation), or multiple combinations of these routes.

[0093] In these formulations, they can be appropriately combined with a pharmacologically or food and drink acceptable carrier, specifically, sterilized water, saline, buffer solution, medium, vegetable oil, solvent, base, emulsifier, suspension, surfactant, stabilizer, flavor agent, air freshener, excipient, vehicle, antiseptic, binder, diluent, isotonic agent, soothing agent, bulking agent, disintegrant, buffer agent, coating agent, lubricant, colorant, sweetener, thickener, flavor modifier, solubilizer, or other additives.

[0094] In addition, in these formulations, from the viewpoint of e.g., more efficiently exerting antibacterial activity against drug-resistant bacteria and the like in the intestinal tract, particularly in a formulation intended for oral administration, the composition of the present invention may be combined with a composition that enables efficient delivery into the intestinal tract. The

composition capable of such delivery into the intestinal tract is not particularly limited, and a known composition can be appropriately employed, and examples thereof include pH-sensitive compositions, compositions that suppress release to the intestinal tract (such as cellulosic polymers, acrylic acid polymers and copolymers, and vinyl acid polymers and copolymers), bioadhesive compositions that specifically adhere to the intestinal mucosa (such as polymers described in U.S. Pat. No. 6,368,586), protease inhibitor-containing compositions, and compositions specifically degraded by intestinal enzymes).

[0095] In addition, when the antibacterial composition of the present invention is used as a pharmaceutical composition, it may further contain a known substance used for treating, preventing, or ameliorating diseases caused by drug-resistant bacteria and the like (such as other antibacterial agents, anti-inflammatory agents, and immunosuppressants), or may be used in combination with such a substance.

[0096] When the composition of the present invention is used as food and drink, the food and drink may be, for example, a health food, a functional food, a food for specified health use, a food with nutrient function claims, a food with functional claims, a dietary supplement, a food for patients, or animal feed. Specific examples of the food and drink include liquid foods such as fermented beverages, oil-containing products, soups, milk beverages, refreshing beverages, tea beverages, alcoholic beverages, energy drinks, and jelly-like beverages, carbohydrate-containing foods, processed livestock foods, and processed fishery foods; and processed vegetable foods, semi-solid foods, fermented foods, confectionery, retort products, microwave-compatible foods, and the like. It is also possible to list healthy food and drink prepared in the form of powder, granules, tablets, capsules, liquid, paste, or jelly. The food and drink according to the present invention can be produced by a production technique known in the art. In the food and drink, components (such as nutrients) effective for ameliorating or preventing diseases caused by drug-resistant bacteria and the like may be added. In addition, it may be a multifunctional food and drink by combining it with other ingredients or other functional foods that exhibit functions other than the amelioration and the like.

[0097] The product (pharmaceutical product, quasi-drug, food and drink, reagent) of the composition of the present invention or the instruction manual thereof may be provided with an indication that it exhibits antibacterial activity against drug-resistant bacteria and the like, or is used for treating, ameliorating, or preventing diseases caused by the drug-resistant bacteria and the like. Further, in relation to the food and drink, the product or the like of the composition of the present invention may be labeled with a health function as a food with health claims (food for specified health use, food with nutrient function claims, food with functional claims) so that the form, target, and the like can be distinguished from general foods. Here, "the product or the instruction manual is labeled" means that a label is attached to the main body, container, packaging, or the like of the product, or a label is attached to the instruction manual, package insert, promotional material, other printed matter, or the like that discloses product information. Further, the composition of the present invention may be in the form of a kit.

[0098] Further, as described above, a pharmaceutical composition can be produced by a known formulation technique using the intestinal bacterium and the like of the present invention. Therefore, the present invention also provides the use of the intestinal bacterium and the like of the present invention for producing a pharmaceutical composition for treating, ameliorating, or preventing diseases caused by drug-resistant bacteria and the like.

<Treatment Method and the Like>

[0099] The present invention also provides a method for treating, ameliorating, or preventing diseases caused by drug-resistant bacteria and the like in a target, wherein the target is allowed to ingest the above-mentioned antibacterial composition or pharmaceutical composition, or the above-mentioned intestinal bacteria as an active ingredient thereof (hereinafter also collectively referred to as "the pharmaceutical composition and the like of the present invention or the active ingredient

and the like thereof").

[0100] In the present invention, the "drug-resistant bacterium" means a bacterium that has resistance to antibacterial agents (such s antibiotics) and the drugs are ineffective or difficult to be effective. Further, the drug may be one drug or multiple drugs. That is, the drug-resistant bacteria according to the present invention also include multidrug-resistant bacteria. Such bacteria are not particularly limited, but examples thereof include carbapenem-resistant Enterobacteriaceae (CRE, KPC-2-producing *Klebsiella pneumoniae*, and the like), vancomycin-resistant enterococci (VRE, bacteria with vancomycin-resistant gene (vanA), and the like), *Clostridium difficile*, and *Campylobacter jejuni*. More specific examples include *Klebsiella pneumoniae* (ATCC BAA-1705), *Enterococcus faecium* (Orla-Jensen) Schleifer and Kilpper-Balz (ATCC 700221), *Clostridioides difficile* (Prevot) Lawson et al. (ATCC 43255, bacterial strain designation: VPI 10463), *Clostridioides difficile* (Prevot) Lawson et al. (ATCC BAA-1382, bacterial strain designation: 630), and *Campylobacter jejuni* 81-176 (ATCC BAA2151).

[0101] The "diseases caused by drug-resistant bacteria" include infectious diseases caused by drug-resistant bacteria. They also include diseases associated with the infection. Examples of such diseases include respiratory infectious diseases such as sepsis, peritonitis, meningitis, gastroenteritis, and pneumonia, urinary tract infectious diseases, surgical site infectious diseases, soft tissue infectious diseases, and medical device-related infectious diseases (such as medical device-related bloodstream infectious diseases).

[0102] In the present invention, the "pro-inflammatory bacterium" means a bacterium that induces inflammation in the intestinal tract, and examples thereof include adherent-invasive *Escherichia coli* (AIEC). More specifically, AIEC LF82 can be mentioned.

[0103] The "diseases caused by pro-inflammatory bacteria" include diseases caused or involved in inflammation caused by the bacteria. Examples of such diseases include inflammatory bowel diseases (including chronic inflammatory bowel diseases such as Crohn's disease, ulcerative colitis, and inflammatory bowel disease).

[0104] The pharmaceutical composition and the like of the present invention or the active ingredient and the like thereof can be used for animals including humans, but there are no particular restrictions on animals other than humans, and various domestic animals, poultry, pets, laboratory animals, and the like can be targeted.

[0105] In addition, the ingestion target of the intestinal bacterium and the like of the present invention includes animals carrying the drug-resistant bacteria and the like, regardless of the onset of diseases caused by the drug-resistant bacteria and the like. From the viewpoint of prevention, an animal that does not possess the bacteria or is suspected of possessing them may be allowed to ingest the pharmaceutical composition and the like of the present invention or the active ingredient and the like thereof.

[0106] The method for ingesting the pharmaceutical composition and the like of the present invention or the active ingredient and the like thereof is not particularly limited, and may be oral administration or parenteral administration (for example, administration into the intestinal tract), and in the case of oral administration, from the viewpoint of further improving the effects of the pharmaceutical composition and the like of the present invention or the active ingredient and the like thereof, it is preferable that the ingestion target of the pharmaceutical composition and the like of the present invention or the active ingredient and the like thereof has reduced production of gastric acid by ingestion of a proton pump inhibitor (PPI) or the like.

[0107] In addition, in the case of ingesting the pharmaceutical composition and the like of the present invention or the active ingredient and the like thereof, the amount ingested can be appropriately selected by those skilled in the art according to the target's age, weight, disease symptoms, health conditions, composition type (such as pharmaceutical product, and food and drink), ingestion method, and the like.

[0108] Preferable embodiments of the antibacterial composition and the pharmaceutical

composition as well as the therapeutic method and the like of the present invention have been described above, but the present invention is not limited to the above embodiments.

[0109] As shown in Examples described later, regarding the ability to suppress intestinal colonization possessed by the Th1 cell-inducible bacterium *Klebsiella* 2H7 strain (Kp2H7), the present inventors have succeeded in selecting 18 strains capable of exerting the same level of suppression ability as the 37 strains of intestinal bacteria derived from healthy subject #F. Therefore, the present invention may also provide the following aspects with respect to antibacterial compositions and pharmaceutical compositions as well as treatment methods and the like.

[0110] <1> An antibacterial composition against Th1 cell-inducible bacteria, containing an intestinal bacterium as an active ingredient, wherein the intestinal bacterium is at least one bacterium which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 69, 80, 85 to 92, 94, 96, 98 to 101, 103, and 105 or a base sequence having at least 90% identity to the base sequence.

[0111] <2> The antibacterial composition according to <1>, which is a pharmaceutical composition.

[0112] <3> The antibacterial composition according to <1> or <2>, which is a pharmaceutical composition for treating, ameliorating, or preventing diseases caused by Th1 cells.

[0113] <4> A method for suppressing proliferation or activation of Th1 cells in a target, a method for suppressing immunity in the target, or a method for treating, ameliorating, or preventing diseases caused by Th1 cells in the target, including allowing the target to ingest the antibacterial composition according to any one of <1> to <3> or at least one bacterium which includes a DNA composed of a base sequence set forth in any of SEQ ID NOs: 69, 80, 85 to 92, 94, 96, 98 to 101, 103, and 105 or a base sequence having at least 90% identity to the base sequence.

[0114] The "Th1 cell-inducible bacterium" according to the present invention is a bacterium that normally exists in the human oral cavity, and induces the proliferation or activation of Th1 cells by colonizing in the intestinal tract. It is preferably a bacterium that belongs to *Klebsiella*, more preferably belonging to *Klebsiella pneumoniae* or *Klebsiella aeromobilis*, and induces the proliferation or activation of Th1 cells in the intestinal tract. Further, the "Th1 cell-inducible bacterium" is preferably a bacterium that easily colonizes in an intestinal environment in which the diversity has changed as compared with the healthy state due to the administration of an antibacterial agent. It is also a bacterium that easily colonizes in an intestinal environment in which the diversity has changed as compared with the healthy state due to colitis or the like.

[0115] For an example of the "Th1 cell-inducible bacterium," PTL 1 can be referred to, and typical examples include Kp2H7 strain, Ka11E12 strain, 34E1 strain, BAA-1705 strain, 700603 strain, and 40B3 strain belonging to *Klebsiella*. Among these, the Kp2H7 strain or the Ka11E12 strain is more preferable, and the Kp2H7 strain is particularly preferable. See Table 6 for details of these bacteria. TABLE-US-00007 TABLE 6 Bacterial Name Supplier Information from Supplier Registry number KCTC2242 KCTC http://koto.kribb.re.kr/English/_ NCBI Taxonomy ID: 1049565 SearchView.aspx?sn=2242 2552 ATCC https://www.atcc.org/Products/ NCBI Taxonomy ID: 507522 All/BAA-2552.aspx KP-1 — — NCBI Taxonomy ID: 1365186 700721 ATCC https://www.atcc.org/Products/ NCBI Taxonomy ID: 272620 All/700721.aspx 13882 JCM https://www.atcc.org/Products/ NCBI Taxonomy ID: 1913574 All/13882.aspx 40B3 — — SAMD00083913 34E1 — — SAMD00083911 1705 ATCC https://www.atcc.org/Products/ NCBI Taxonomy ID: 1276652 All/BAA-1705.aspx 11E12 — — SAMD00083912 700603 ATCC https://www.atco.org/Products/ NCBI Taxonomy ID: 1276653 All/700603.aspx 2H7 — — SAMD00083910

[0116] In addition, examples of the "Th1 cell-inducible bacterium" of the present invention include a bacterium which contains a DNA composed of a nucleotide sequence having 90% or more (91% or more, 92% or more, 93% or more, 94% or more, 95% or more, 96% or more, 97% or more, 98%

or more, and 99% or more) identity to the nucleotide sequence encoding the 16S rRNA of Kp2H7 strain, Ka11E12 strain, 34E1 strain, BAA-1705 strain, 700603 strain, or 40B3 strain, and also include a bacterium which contains a DNA composed of a nucleotide sequence having 70% or more (preferably 80% or more, more preferably 85% or more, further preferably 90% or more, and more preferably 94% or more (for example, 95% or more, 96% or more, 97% or more, 98% or more, and 99% or more)) homology or identity to the nucleotide sequence specific to Kp2H7 strain, Ka11E12 strain, 34E1 strain, BAA-1705 strain, 700603 strain, or 40B3 strain.

[0117] In the present invention, the "Th1 cell" is a subgroup of CD4 positive helper T-cells (Th cells) and means a cell that enhances cell-mediated immunity. In addition, the meaning of "activation of Th1 cells" includes the production of Th1 cytokines (such as IFN-γ) by the cells, induction of macrophages by the cytokines, activation of cells such as cytotoxic T-cells (CTL), and enhancement of cell-mediated immunity by the activation. Further, the meaning of "induction of the proliferation or activation of Th1 cells" also includes induction of differentiation from naive T-cells to Th1 cells which leads to proliferation or activation of Th1 cells.

[0118] The effect of inducing proliferation or activation of Th1 cells in the intestinal tract can be evaluated by quantitatively detecting Th1 cell-specific markers (such as CD4 and IFN-γ). Such quantitative detection can be performed by a known method, and can be performed by methods for detection using antibodies (immunological methods) such as flow cytometry, imaging cytometry, ELISA method, radioimmunoassay, immunohistochemical staining, immunoprecipitation, immunoblotting, and antibody array analysis.

[0119] Regarding whether or not any bacterium or the like has an effect of inducing proliferation or activation of Th1 cells in the intestinal tract, for example, it can be determined that the bacterium or the like has an effect of inducing proliferation or activation of Th1 cells in the intestinal tract when the proportion of IFN-γ.sup.+ cells in CD4.sup.+TCRβ.sup.+ T-cells in the intestinal tract detected by flow cytometry is 10% or more (it is preferable to determine, when the proportion is 25% or more, that the bacterium or the like has an effect of inducing proliferation or activation of Th1 cells in the intestinal tract, and it is more preferable to determine, when the proportion is 30% or more, that the bacterium, substance, or the like has an effect of inducing proliferation or activation of Th1 cells in the intestinal tract).

[0120] In the present invention, the "diseases caused by Th1 cells" mean diseases induced by proliferation or activation of Th1 cells, and examples thereof include inflammatory bowel diseases (including chronic inflammatory bowel diseases such as Crohn's disease, ulcerative colitis, and inflammatory bowel disease), type 1 diabetes, rheumatoid arthritis, experimental autoimmune encephalomyelitis (EAE), multiple sclerosis, autoimmune diseases such as systemic lupus erythematosus, and chronic inflammatory diseases. In addition, the "immunity" suppressed in the present invention includes not only mucosal immunity (such as intestinal immunity) but also systemic immunity. Furthermore, not only cell-mediated immunity but also humoral immunity is included.

[0121] In addition, when the antibacterial composition of the present invention is used as a pharmaceutical composition, it may further contain known substances (such as anti-inflammatory agents and immunosuppressants) used for the treatment, prevention, or amelioration of diseases caused by Th1 cells, or may be used in combination with such substances.

[0122] Note that for other phrases in <1> to <4>, refer as appropriate to the above-mentioned <Antibacterial Composition and Pharmaceutical Composition> and <Treatment Method and the Like>.

<Composition for Examining Diseases Caused by Drug-Resistant Bacteria and the Like>

[0123] In the present invention, the presence of intestinal bacteria that can suppress the colonization and the like of drug-resistant bacteria and the like in the intestinal tract has been clarified. Therefore, by detecting the presence of the intestinal bacteria, it becomes possible to examine diseases caused by drug-resistant bacteria and the like.

[0124] Therefore, the present invention provides a composition for examining diseases caused by the following drug-resistant bacteria and the like.

[0125] A composition for examining diseases caused by drug-resistant bacteria and the like, containing an antibody that specifically recognizes the intestinal bacterium and the like of the present invention.

[0126] A composition for examining diseases caused by drug-resistant bacteria and the like, containing a polynucleotide for detecting a nucleotide sequence specific to the drug-resistant bacteria and the like of the present invention.

[0127] In the present invention, the "antibody that specifically recognizes the intestinal bacterium and the like of the present invention" may be a polyclonal antibody, a monoclonal antibody, or a functional fragment of antibody (for example, Fab, Fab′, F(ab′)2, variable region fragment (Fv), disulfide bond Fv, single chain Fv (scFv), sc (Fv)2, diabody, multispecific antibody, or a polymer thereof) as long as it can specifically recognize the bacterium. If the antibody of the present invention is a polyclonal antibody, it can be obtained by immunizing an host animal with an antigen (peptide, polynucleotide, sugar chain, lipid, or the like derived from the intestinal bacterium and the like of the present invention) and purifying it from its antiserum by a conventional means (such as salting-out, centrifugation, dialysis, or column chromatography). In addition, the monoclonal antibody can be produced by a hybridoma method or a recombinant DNA method.

[0128] Further, as the antibody used for the examination of the present invention, it is possible to use an antibody bound with a labeling substance. By detecting the labeling substance, it is possible to directly measure the amount of antibody bound to the intestinal bacterium and the like of the present invention or a substance derived from the bacterium. The labeling substance is not particularly limited as long as it can bind to an antibody and can be detected by a chemical or optical method, and examples thereof include fluorescent dyes (such as GFP), enzymes (such as HRP), and radioactive substances.

[0129] In addition to the antibody component, the examination composition of the present invention may contain additional components acceptable as a composition. Examples of the additional components include carriers, excipients, disintegrants, buffer agents, emulsifiers, suspensions, stabilizers, preservatives, antiseptics, saline, labeling substances, and secondary antibodies. In addition to the above examination compositions, it is possible to combine substrates required for detection of labeling substances, positive controls and negative controls, buffer solutions used for diluting or washing the sample, tubes or plates used for the reaction of the sample with the antibody of the present invention, and the like, or use as an examination kit for diseases caused by drug-resistant bacteria and the like is possible. In addition, when an unlabeled antibody is used as an antibody standard sample, it is possible to combine a labeled substance (such as secondary antibody, protein G, or protein A) that binds to the antibody. Further, the kit for examining diseases caused by drug-resistant bacteria and the like can include an instruction manual for use of the kit.

[0130] Further, the examination composition of the present invention can be combined with an apparatus for detecting the antibody of the present invention. Examples of the apparatus include a flow cytometry apparatus and a microplate reader.

[0131] In the present invention, the "polynucleotide for detecting a nucleotide sequence specific to the intestinal bacterium and the like of the present invention" is not particularly limited as long as the sequence specific to the bacterium is detected, and examples thereof include polynucleotides according to any of the following (a) and (b), each having a chain length of at least 15 nucleotides.

[0132] (a) A polynucleotide that is a pair of primers designed to sandwich the specific nucleotide sequence, and [0133] (b) A polynucleotide that is a primer or probe that hybridizes to a nucleotide sequence containing the specific nucleotide sequence.

[0134] The polynucleotide of the present invention has a base sequence complementary to the

nucleotide sequence of the intestinal bacterium and the like of the present invention. Here, "complementary" does not have to be perfectly complementary as long as it hybridizes. These polynucleotides usually have 80% or more, preferably 90% or more, more preferably 95% or more, and particularly preferably 100% homology to the nucleotide sequence.

[0135] The "chain length" of the polynucleotide of the present invention, when used as a primer, is usually 15 to 100 nucleotides, preferably 17 to 30 nucleotides, and more preferably 20 to 25 nucleotides. In addition, when used as a probe, it is usually 15 to 1000 nucleotides, and preferably 20 to 100 nucleotides.

[0136] The polynucleotide of the present invention may be a DNA or RNA, or may have its nucleotide replaced with an artificial nucleic acid such as LNA (registered trademark, crosslinked nucleic acid), ENA (registered trademark, 2′-O, 4′-C-Ethylene-bridged nucleic acids), GNA (glycerol nucleic acid), TNA (threose nucleic acid), or PNA (peptide nucleic acid) in part or all of it.

[0137] Note that the polynucleotide of the present invention can be chemically synthesized using a commercially available nucleotide automatic synthesizer or the like. Further, as the polynucleotide used for the examination of the present invention, it is possible to use a polynucleotide bound with a labeling substance. The labeling substance is not particularly limited as long as it can bind to a polynucleotide and can be detected by a chemical or optical method, and examples thereof include fluorescent dyes (such as DEAC, FITC, R6G, TexRed, and Cy5) as well as dyes such as DAB (chromogen), enzymes, and radioactive substances in addition to fluorescent dyes.

[0138] In addition to the above-mentioned polynucleotide, the examination composition of the present invention may contain additional pharmacologically acceptable components. Examples of the additional components include buffer agents, emulsifiers, suspensions, stabilizers, antiseptics, and saline.

[0139] In addition to the above examination compositions, it is possible to combine standard samples such as substrates required for detection of labeling substances added to the polynucleotide, positive controls and negative controls, and buffer solutions used for diluting or washing the sample, thereby combining tubes or plates used for the reaction of the sample with the polynucleotide of the present invention, or use as an examination kit for diseases caused by drug-resistant bacteria and the like is possible. Moreover, the kit for examining diseases caused by drug-resistant bacteria and the like can include an instruction manual for use of the kit.

[0140] In addition, the examination composition of the present invention can be combined with an apparatus for detecting a nucleotide sequence specific to the intestinal bacterium and the like of the present invention. Examples of the apparatus include a thermal cycler, a sequencer, and a microarray.

[0141] Furthermore, the present invention also provides a method examining diseases caused by drug-resistant bacteria and the like using the above-mentioned antibody, polynucleotide, or examination composition. Specifically, the present invention provides a method for examining diseases caused by drug-resistant bacteria and the like, including the steps of contacting the antibody, polynucleotide, or examination composition with a sample isolated from a subject, and detecting the presence or absence of an intestinal bacterium and the like of the present invention in the intestinal tract by the contact.

[0142] The subject is not particularly limited, and examples thereof include animals such as humans suspected of suffering from a disease caused by drug-resistant bacteria and the like. Further, the sample isolated from the subject is not particularly limited, but a fecal sample of a subject, a culture thereof, or a polypeptide, polynucleotide, sugar chain, lipid or the like extracted therefrom is preferably used in the method of the present invention.

[0143] As the method for detecting the presence or absence of an intestinal bacterium and the like of the present invention by contacting the sample with the antibody of the present invention or an examination composition containing the same, examples thereof include methods for detection

using antibodies (immunological methods) such as ELISA method, immunoblotting, antibody array analysis, immunohistochemical staining, flow cytometry, imaging cytometry, radioimmunoassay, and immunoprecipitation.

[0144] Further, as the method for detecting the presence or absence of an intestinal bacterium and the like of the present invention by contacting the sample with the polynucleotide of the present invention or an examination composition containing the same, it is possible to use PCR (RT-PCR, real-time PCR, and quantitative PCR), DNA microarray analysis, Northern blotting, 16s rRNA sequencing, next-generation sequencing (synthetic sequencing (sequencing-by-synthesis, for example, sequencing by Solexa Genome Analyzer or Hiseq (registered trademark) 2000 manufactured by Illumina), pyrosequencing (for example, sequencing by a sequencer GSLX or FLX manufactured by Roche Diagnostics (454) (so-called 454 sequencing)), and ligase reaction sequencing (for example, sequencing by SoliD (registered trademark) or 5500x1 manufactured by Life Technologies)), bead array method, in situ hybridization, dot blotting, RNase protection assay, mass spectrometry, genomic PCR method, and Southern blotting.

[0145] In the present invention, the "examination" of a disease caused by drug-resistant bacteria and the like includes not only the presence or absence of the onset of the disease but also the risk of the onset of the disease, and if the presence of an intestinal bacterium and the like of the present invention is detected in the intestinal tract by the above-mentioned method, it can be determined that a disease caused by drug-resistant bacteria and the like has not developed or the risk of developing the disease is low.

[0146] Diagnosis of a disease caused by drug-resistant bacteria and the like in a subject is usually made by a doctor (including a person who has been instructed by a doctor), but the data obtained by the method of the present invention is useful for diagnosis by a doctor. Therefore, the method of the present invention can also be expressed as a method of collecting and presenting data useful for diagnosis by a doctor.

[0147] Further, the present invention can also provide a companion diagnostic method using the above-mentioned examination method and a drug thereof. That is, the present invention also provides the following.

[0148] A method for determining the effectiveness of the pharmaceutical composition and the like of the present invention or the active ingredient and the like thereof in the treatment, amelioration, or prevention of diseases caused by drug-resistant bacteria and the like, including the steps of contacting the antibody, polynucleotide, or examination composition with a sample isolated from a subject, and detecting the presence or absence of the intestinal bacteria and the like by the contact, wherein in the step, when the presence of the bacteria is not detected, it is determined that the pharmaceutical composition and the like of the present invention or the active ingredient and the like thereof in the subject is highly effective in treating, ameliorating, or preventing the disease.

[0149] A method for treating, ameliorating, or preventing diseases caused by drug-resistant bacteria and the like, including a step of allowing a patient determined by the determination method to have high effectiveness of the pharmaceutical composition and the like of the present invention or the active ingredient and the like thereof to ingest the pharmaceutical composition and the like or the active ingredient and the like thereof.

[0150] A composition for treating, ameliorating, or preventing diseases caused by drug-resistant bacteria and the like, containing the intestinal bacterium and the like of the present invention as an active ingredient, wherein the composition is ingested by a subject determined to have high effectiveness by the determination method.

<Method for Screening Intestinal Bacteria Having Antibacterial Activity Against Drug-Resistant Bacteria and the Like in Intestinal Tract>

[0151] It has also been clarified for the first time by the present inventors that among intestinal bacteria, there are bacteria that suppress the colonization of drug-resistant bacteria and the like in the intestinal tract. Therefore, the present invention provides a method for screening intestinal

bacteria having antibacterial activity against drug-resistant bacteria and the like, including the following steps.

[0152] A step of allowing a non-human germ-free animal to ingest drug-resistant bacteria and the like and test intestinal bacteria in the intestinal tract, a step of detecting the drug-resistant bacteria and the like in the intestinal tract of the non-human germ-free animal, and a step of determining the test intestinal bacteria as intestinal bacteria having antibacterial activity against the drug-resistant bacteria and the like when the number of bacteria detected in the step is reduced as compared with the case where the test intestinal bacteria are not ingested.

[0153] The "drug-resistant bacteria and the like" are as described above. The "non-human germ-free animal" means a non-human animal that is born and grown under germ-free conditions. Examples of non-human animals include, but are not limited to, mice, rats, monkeys, pigs, cows, horses, sheep, goats, chickens, ducks, ostriches, domestic ducks, dogs, cats, rabbits, and hamsters. Moreover, in these animals, mice are preferably used.

[0154] The test intestinal bacteria to be ingested by non-human germ-free animals may be any bacteria present in the intestines of animals, examples of such animals include humans and non-human animals (such as mice, rats, monkeys, pigs, cows, horses, sheep, goats, chickens, ducks, ostriches, domestic ducks, dogs, cats, rabbits, and hamsters). In addition, the test intestinal bacteria to be ingested by non-human germ-free animals may be isolated intestinal bacteria, and examples thereof include a sample containing intestinal bacteria (for example, a fecal sample of the animal or a culture thereof).

[0155] Further, the method for "ingesting" the test intestinal bacteria and drug-resistant bacteria and the like to a non-human animal is not particularly limited, and is usually performed by oral administration, but may be parenteral administration (for example, administration into the intestinal tract). In addition, the test intestinal bacteria and drug-resistant bacteria and the like may be ingested at the same time, the drug-resistant bacteria and the like may be ingested by the non-human animal after the test intestinal bacteria are ingested by the animal, or the test intestinal bacteria may be ingested by the non-human animal after the drug-resistant bacteria and the like are ingested by the animal.

[0156] The "detection" of drug-resistant bacteria and the like in the intestinal tract can be performed by detecting a nucleotide sequence specific to the drug-resistant bacteria and the like. Examples of the detection method include PCR (RT-PCR, real-time PCR, and quantitative PCR), DNA microarray analysis, Northern blotting, 16s rRNA sequencing, next-generation sequencing (synthetic sequencing (sequencing-by-synthesis, for example, sequencing by Solexa Genome Analyzer or Hiseq (registered trademark) 2000 manufactured by Illumina), pyrosequencing (for example, sequencing by a sequencer GSLX or FLX manufactured by Roche Diagnostics (454) (so-called 454 sequencing)), and ligase reaction sequencing (for example, sequencing by SoliD (registered trademark) or 5500x1 manufactured by Life Technologies)), bead array method, in situ hybridization, dot blotting, RNase protection assay, mass spectrometry, genomic PCR method, and Southern blotting.

[0157] Further, the "detection" of drug-resistant bacteria and the like in the intestinal tract can be performed, for example, by detecting an amino acid sequence specific to the drug-resistant bacteria and the like. Examples of the detection method include methods for detection using antibodies (immunological methods) such as ELISA method, immunoblotting, antibody array analysis, immunohistochemical staining, flow cytometry, imaging cytometry, radioimmunoassay, and immunoprecipitation. The timing of detection is not particularly limited, and those skilled in the art can appropriately adjust the timing according to the type and the like of animal to be used.

[0158] Note that if the screening method of the present invention fails to select intestinal bacteria having antibacterial activity against drug-resistant bacteria and the like in a single trial, an intestinal sample containing the obtained bacteria is ingested by a new non-human germ-free animal as the next test intestinal bacteria, and the above-mentioned screening is performed multiple times,

making it possible to isolate the intestinal bacteria having the antibacterial activity.

EXAMPLES

Example 1

[0159] As shown in the upper part of FIG. **1**, germ-free mice were administered with *Klebsiella* 2H7 strains (Kp2H7), and one week later, fecal samples of healthy volunteers were administered.

[0160] Specifically, the germ-free mice used were C57BL/6N at 4 to 8 weeks of age (CLEA Japan, Inc.), which were kept in a rearing vinyl isolator (germ-free isolator) (manufactured by ICM Co., Ltd.; ICM-1B) under the conditions of free-drinking water and feeding for one week or longer to acclimate them to the environment. The age at the start of the experiment was 8 to 14 weeks of age. The same applies to the other Examples in the present specification.

[0161] A bacterial solution of *Klebsiella* was placed in an LB liquid medium and cultured overnight at 37° C. to adjust the OD value to 1.2 (equivalent to $1*10^9$ CFU/mL), and 200 μL/individual (equivalent to $2*10^8$ CFU/individual) of bacterial solution was administered into the stomach of mice using a sonde.

[0162] For the fecal samples, feces provided by Japanese healthy subject volunteers (#A, #F, #I, #J, and #K) were diluted 5-fold by weight with a glycerol PBS solution (final concentration of glycerol: 20% by volume), which was filtered through a 100 μm diameter filter, and the resultant was stored as a stock solution at −80° C. At the time of fecal administration, the stock solution was diluted 10-fold with PBS in an anaerobic chamber, and administered in an amount of 200 μL/individual into the stomach of each mouse using a sonde.

[0163] Mouse fecal samples were dissolved in a solution of glycerol (final concentration and 20%) EDTA (final concentration 10 mM) mixed in PBS at a proportion of 50 mg feces/mL. The fecal lysate was seeded after appropriate dilution in DHL medium containing 50 mg/L ampicillin and 50 mg/L spectinomycin, and after culturing at 37° C. overnight, the number of colonies was counted and the number of CFUs per 1 g of feces was calculated.

[0164] In the other Examples in the present specification, bacterial solution of *Klebsiella* and feces were administered in the same manner, and CFUs were counted in the same manner.

[0165] As a result, as shown in the lower part (graph) of FIG. **1**, five types of feces were used, and a significant decrease in the bacterial amount of Kp2H7 was observed in all the samples.

(Example 2) Isolation of Bacteria from Feces of Healthy Subject Volunteers

[0166] Each of the frozen fecal samples derived from feces of healthy subjects F, K, and I (feces F, feces K, and feces I) prepared in Example 1 was thawed at room temperature, diluted with PBS, and cultured in an agar plate of EG medium, modified GAM agar medium (Nissui Pharmaceutical Co., Ltd.; 05426), REINFORCED CLOSTRIDIAL AGAR (RCM AGAR) (Thermo Fisher Scientific Inc; CM0151), or Schaedler blood medium (manufactured by Wako; 517-45805) under an anaerobic environment of at 37° C. and 10% CO.sub.2, and the colonies formed were isolated. From feces F, 37 strains were isolated, 42 strains were isolated from feces I, and 47 strains were isolated from feces K. After that, the feces K was isolated again, and finally 68 strains were isolated from the feces K.

[0167] For the isolated bacteria, the gene sequence was analyzed and the bacterial species was estimated by 16S rDNA analysis by the Sanger method. Sequence analysis was performed using 3130 DNA Analyzer manufactured by Thermo Fisher Scientific and a primer set having the following sequences.

27 Forward-mod: 5′-AGRGTTTGATYMTGGCTCAG-3′ (SEQ ID NO: 148)

1492 Reverse: 5′-GGYTACCTTGTTACGACTT-3′ (SEQ ID NO: 149)

[0168] Here, R: A or G, Y: C or T, and M: A or C.

[0169] Furthermore, the genome sequences of the 37 bacterial strains derived from feces F were determined using a next-generation sequencer. That is, genome sequencing was performed using MiSeq manufactured by Illumina and Sequel manufactured by Pacific Biosciences, and all genome sequences were obtained by hybrid assembly using Unicycler. By extracting the 16S rRNA

sequence using RNAmmer for each of these genome sequences, a more accurate sequence was obtained, including both terminal sequences that could not be determined by the 16S rDNA sequence determined by the Sanger method.

[0170] Tables 1 to 4 show the results of this analysis. In addition, FIG. **2** shows the results of 16S meta-analysis of three types of feces derived from donors F, I, and K.

Example 3

[0171] As shown in the upper part of FIG. **3**, Kp2H7 was administered to germ-free mice, and one week later, mixed isolated bacteria (37 strains derived from feces F (F37mix), 42 strains derived from feces I (I42mix), and 47 strains derived from feces K (K47mix)) or feces I were administered.

[0172] The isolated bacteria were cultured in mGAM liquid medium, EG medium, or CM0149 medium at 37° C. in an anaerobic chamber for 24 to 48 hours, and mixed. The mixed solution was concentrated 5 times, and 200 μL/individual (equivalent to total bacterial amount $1*10^9$ CFU/animal) of bacterial solution was administered into the stomach using a sonde. The mixed isolated bacterial strains in the following Examples were also administered in the same manner.

[0173] As a result, as shown in the lower part (graph) of FIG. **3**, it was clarified that the 37 strains derived from feces F had the same activity as feces I in terms of the ability to eliminate *Klebsiella* from the mouse intestinal tract.

Example 4

[0174] As shown in the upper part of FIG. **4**, Kp2H7 was administered to germ-free mice, and one week later, the mixed isolated bacteria (37 strains derived from feces F and 68 strains derived from feces K) were administered. As a result, as shown in the lower part (graph) of FIG. **4**, 37 strains derived from feces F and 68 strains derived from feces K equally eliminated *Klebsiella* from the mouse intestinal tract.

Example 5

[0175] As shown in the upper part of FIG. **5**, Kp2H7 was administered to germ-free mice, and one week later, mixed isolated bacteria (F37mix) were administered, and 200 mg/L of ampicillin was administered by drinking water after the administration of the isolated bacteria. In order to investigate the change in the bacterial amount of each of the administered bacteria, PCR was performed using a specific primer of each of the bacteria. Table 7 shows the primers used in the analysis.

TABLE-US-00008 TABLE 7 Forward SEQ ID Reverse SEQ ID Primer Name NO: Primer Name NO: f01 f01_42H6_Fw1 150 f01_42H6_Rv1 151 f02 f02_42I8_Fw1 152 f02_42I8_Rv3 153 f05 f05_43D4_Fw4 154 f05_43D4_Rv2 155 f06 f06_42F4_Fw1 156 f06_42F4_Rv1 157 f07 f07_42J6_Fw1 158 f07_42J6_Rv1 159 f08 f08_42B5_Fw1 160 f08_42B5_Rv1 161 f09 f09_43F1_Fw1 162 f09_43F1_Rv1 163 f10 f10_43J3_Fw1 164 f10_43J3_Rv1 165 f12 f12_42H4_Fw1 166 f12_42H4_Rv1 167 f13 f13_42H8_Fw1 168 f13_42H8_Rv1 169 f14 f14_42L4_Fw1 170 f14_42L4_Rv1 171 f16 f16_43M1_Fw2 172 f16_43M1_Rv2 173 f17 f17_42I7_Fw3 174 f17_42I7_Rv3 175 f18 f18_42I2_Fw5 176 f18_42I2_Rv5 177 f19 f19_43G2_Fw2 178 f19_43G2_Rv2 179 f20 f20_43G1_Fw2 180 f20_43G1_Rv2 181 f21 f21_42A8_Fw2 182 f21_42A8_Rv6 183 f22 f22_43C3_Fw2 184 f22_43C3_Rv2 185 f24 f24_42I4_Fw1 186 f24_42I4_Rv1 187 f25 f25_42J1_Fw2 188 f25_42J1_Rv2 189 f26 f26_42K2_Fw1 190 f26_42K2_Rv1 191 f27 f27_42J5_Fw2 192 f27_42J5_Rv2 193 f28 f28_43A3_Fw1 194 f28_43A3_Rv1 195 f29 f29_43J8_Fw2 196 f29_43J8_Rv2 197 f30 f30_43A5_Fw1 198 f30_43A5_Rv1 199 f31 f31_43J5_Fw1 200 f31_43J5_Rv2 201 f32 f32_42A7_Fw1 202 f32_42A7_Rv2 203 f33 f33_43N2_Fw1 204 f33_43N2_Rv1 205 f34 f34_43K4_Fw1 206 f34_43K4_Rv1 207 f35 f35_42L8_Fw2 208 f35_42L8_Rv2 209 f37 f37_42G1_Fw3 210 f37_42G1_Rv4 211

[0176] As a result, as shown in the lower part of FIG. **5**, the bacterial amount of *Klebsiella* was transiently increased by the administration of ampicillin, but decreased again thereafter.

[0177] In addition, the change over time of the abundance ratio of each isolated bacterium in the total bacterial amount was analyzed together with that of Kp2H7. FIGS. **6**A**1**-**6**A**4**, **6**B**1**-**6**B**4**, **6**C**1**-**6**C**4**, **6**D**1**-**6**D**4**, **6**E**1**-**6**E**4**, **6**F**1**-**6**F**4**, **6**G**1**-**6**G**3**, and **6**H**1**-**6**H**3** show the results obtained. Furthermore, the bacterial amount of *Klebsiella* and the Spearman's rank correlation coefficient of each bacterium were calculated and arranged in order of high positive correlation. FIG. **7** shows the results obtained.

[0178] As shown in FIG. **7**, it was found that the strains belonging to Bacteroidetes moved regardless of the movement of *Klebsiella*. On the other hand, many of the strains having a negative correlation belonged to the genus *Furmicutes.*

Example 6

[0179] As shown in FIG. **9**, 37 the strains derived from feces F were divided into 8 strains belonging to Bacteroidetes (F8mix) and the 29 other bacterial strains (F29mix), and mixed respectively, and after Kp2H7 was colonized in germ-free mice, the mixed isolated bacteria were administered. Note that the phylogenetic trees shown in FIGS. **9**, **10**, and **12** were prepared by the Neighbor-joining method using MEGA X with the DNA base sequence of the 16S rDNA analysis result of the isolated bacteria by the Sanger method.

[0180] As a result, as shown in FIG. **8**, F37mix and F29mix equally eliminated *Klebsiella* from the mouse intestinal tract. On the other hand, in the F8mix administration group belonging to Bacteroidetes, the bacterial amount of *Klebsiella* did not change, suggesting that F8mix is not related to the elimination of *Klebsiella.*

Example 7

[0181] From the 37 strains, 18 strains were selected by excluding strains belonging to Bacteroidetes, strains duplicate at the 16S rDNA level, strains disappeared by ampicillin administration, and strains showing behavior unrelated to *Klebsiella* (see FIG. **10**).

[0182] Then, as shown in the upper part of FIG. **11**, the Kp2H7 strains were administered to germ-free mice, and one week later, mixed isolated bacteria (37 strains derived from feces F (F37mix), 18 strains (F18mix), and 42 strains derived from feces I (I42mix)) were administered.

[0183] As a result, as shown in the lower part (graph) of FIG. **11**, it was clarified that the 18 strains shown in FIG. **10** was also able to exhibit the ability to eliminate *Klebsiella* strains as much as the 37 strains.

Example 8

[0184] The 18 strains shown in FIG. **10** were divided into 4 groups based on the phylogenetic tree (*Blautia, Lachonoclostridum,* other *Firmicutes,* and other Phyla) (see FIG. **12**). Furthermore, these 4 groups were subtracted from the 18 bacterial strains (F18mix) to prepare bacterial strain groups of F15mix (F18mix-other phyla), F12mix (F18mix-Lachnoclostridium), F14mix (F18mix-*Blautia*), F13mix (F18mix-other *Firmicutes*). In addition, a bacterial strain set was also prepared by mixing 13 strains (F13mix (F31-18mix)), which were obtained by removing the duplicated strains from the 37 strains and further removing the above-described 18 strains from the remaining 31 strains. Then, as shown in the upper part of FIG. **13**, Kp2H7 was administered to germ-free mice, and one week later, each isolated bacterium mixed as described above was administered.

[0185] As a result, as shown in the lower part (graph) of FIG. **13**, F18mix best eliminated *Klebsiella*. However, the bacterial amount of *Klebsiella* significantly increased regardless of which group was excluded. In addition, as shown in FIG. **14**, also in terms of the CFU of fecal *Klebsiella* in each administration group at the time of day 28 in the experiment shown in FIG. **13**, F18mix was able to reduce *Klebsiella* statistically significantly by more than any other group excluding F37mix.

[0186] From the above, it was suggested that all of the four groups shown in FIG. **10** were involved in the elimination of *Klebsiella,* and eliminated *Klebsiella* as a community.

[0187] Further, in the experiment shown in FIG. **13**, lymphocytes in the lamina propria of the large intestine were extracted from mice in the F37mix, F18mix, and F31-18mix groups and subjected to analysis by flow cytometry.

[0188] As a result, as shown in FIG. **15**, it was clarified that the proportion of CD4+IFNγ+ cells was high in the F13mix (F31-18mix) group, and that the induction of Th1 cells was suppressed in F37mix and F18mix.

Example 9

[0189] As shown in Example 8, in the experiment of removing the bacteria of each group from F18mix, F15mix obtained by removing the 3 strains of other Phyla had the lowest ability to eliminate *Klebsiella*. Therefore, paying attention to this group, the present inventors prepared groups each obtained by removing any one of these 3 strains (*E. coli*, *Bifidobacterium*, and *Fusobacterium*) from the 18 strains derived from feces F. Then, as shown in the upper part of FIG. **16**, the Kp2H7 strains were administered to germ-free mice, and one week later, isolated bacteria mixed as described above, F18mix, or F15mix were administered.

[0190] As a result, as shown in the lower part (graph) of FIG. **16**, by removing any of the above three strains, an increase of about 1 log in the bacterial amount of *Klebsiella* was observed, suggesting that all of the strains were involved in the elimination of *Klebsiella.*

Example 10

[0191] In order to explore the mechanism of eliminating *Klebsiella* of F37mix, the present inventors focused on whether host immunity was involved in this mechanism. Therefore, as shown in the upper part of FIG. **17**, the *Klebsiella* 2H7 strain was administered to a germ-free Rag2.sup.−/−yc.sup.−/− mouse, MyD88.sup.−/− Triff.sup.−/− mouse, or wild-type mouse, and one week later, mixed F37mix was administered.

[0192] As a result, as shown in the lower part (graph) of FIG. **17**, the same degree of elimination of the *Klebsiella* 2H7 strain by F37mix was observed in all types of mice. This suggests that the major innate immunity and acquired immunity of the host are not involved in the elimination of *Klebsiella.*

[0193] Next, the effect of eliminating the isolated bacterial strains on pathogens and drug-resistant bacteria other than the Kp2H7 strain was evaluated. Note that in the analysis, the primers shown in Table 8 were used as primers specific to the bacteria.

TABLE-US-00009 TABLE 8 Target Bacteria SEQ ID and the Like Primer Name NO: CRE KPC-F684 212 KPC-R860 213 VRE(vanA) VanABF_JCM1998 214 VanAR_JCM1998 215 ESBL ESBL-F_AAC2001 216 ESBL-R_AAC2001 217 LF82-specific pMT gene LF82-F 218 LF82-R 219 *C. jejuni C.jejuni*_F 220 *C.jejuni*_R 221 Universal Universal_Fw_1387 222 Universal_Rv_1492 223 *C. diff* tpi-F 224 tpi-R 225 C. upsaliensis Cupsaliensis_2F 226 Cupsaliensis_2R 227 C.upsaliensis Cupsaliensis_3F 228 Cupsaliensis_3R 229 Kp-P1 khe-Fw 230 khe -RV 231 E.gallinarum Eg_2F 232 Eg_2R 233 P.mirabilis P.mirabillis_NatMicro_F 234 P.mirabillis_NatMicro_R 235

Example 11

[0194] First, as shown in the upper part of FIG. **18**, carbapenem-resistant *Klebsiella* (CRE) was administered to germ-free mice, and one week later, mixed F37mix, K68mix, or I42mix was administered. The results obtained are shown at the lower part of FIG. **18**.

[0195] Note that a bacterial solution of CRE was placed in an LB liquid medium and cultured overnight at 37° C. to adjust the OD value to 1.2 (equivalent to $1*10^{9}$ CFU/mL), and 200 μL/individual (equivalent to $2*10^{8}$ CFU/individual) of bacterial solution was administered into the stomach of mice using a sonde. Regarding the CFU count for CRE, DHL medium containing 30 mg/L ampicillin and 30 mg/L spectinomycin was used as a selective medium and cultured overnight at 37° C. under aerobic conditions.

[0196] In addition, the mice one month after administration of the mixed isolated bacteria were dissected, and the large intestine was fixed with 4% PFA and embedded in paraffin to prepare sliced sections. These sections were stained with hematoxylin solution and eosin solution to observe inflammatory images of the tissues. FIG. **9** shows the obtained results.

[0197] As shown in the lower part (graph) of FIG. **18**, F37mix and K68mix showed equivalent

CRE elimination ability, and I42mix was slightly inferior to them. In addition, as shown in FIG. **19**, no inflammatory findings such as ulceration or infiltration of inflammatory cells were observed in any of the mice. From this, it was clarified that the induction of inflammation in the large intestine was suppressed by administering each of the isolated bacterial mixed groups.

Example 12

[0198] As shown in the upper part of FIG. **20**, vancomycin-resistant *Enterococcus faecium* (VRE) was administered to germ-free mice, and one week later, mixed F37mix, K68mix, or I42mix was administered.

[0199] Note that a bacterial solution of VRE was placed in an LB liquid medium and cultured overnight at 37° C. to adjust the OD value to 1.2 (equivalent to $1*10${circumflex over ( )}9 CFU/mL), and 200 μL/individual (equivalent to $2*10${circumflex over ( )}8 CFU/individual) of bacterial solution was administered into the stomach of mice using a sonde. Regarding the CFU count for VRE, VRE medium (Nippon Becton) was used and cultured overnight at 37° C. under aerobic conditions.

[0200] As a result, as shown in the lower part (graph) of FIG. **20**, K68mix exhibited the highest elimination ability on VRE. In addition, as shown in FIG. **21**, no inflammatory findings such as ulceration or infiltration of inflammatory cells were observed in any of the mice. From this, it was clarified that the induction of inflammation in the large intestine was suppressed by administering each of the isolated bacterial mixed groups.

Example 13

[0201] As shown in the upper part of FIG. **22**, adhesion-invasive *E. coli* (AIEC LF82) was administered to germ-free mice, and one week later, mixed F37mix, K68mix, and I42mix were administered.

[0202] Note that a bacterial solution of AIEC LF82 was placed in an LB liquid medium and cultured overnight at 37° C. to adjust the OD to 1.2 (equivalent to $1*10${circumflex over ( )}9 CFU/mL), and 200 μL/individual (equivalent to $2*10${circumflex over ( )}8 CFU/individual) of bacterial solution was administered into the stomach of mice using a sonde. Regarding the CFU count for AIEC LF82, MacConkey medium containing 1 mg/L cefotaxime was used as a selective medium and cultured overnight at 37° C. under aerobic conditions to calculate the CFU.

[0203] As a result, as shown in the lower part (graph) of FIG. **22**, F37mix had the highest elimination ability on AIEC LF82.

Example 14

[0204] As shown in the upper part of FIG. **23**, ESBL-producing *Klebsiella* (Kp-ESBL) (ATCC 700721) was administered to germ-free mice, and one week later, mixed F37mix, K68mix, I42mix, or feces F was administered.

[0205] Note that a bacterial solution of Kp-ESBL was placed in an LB liquid medium and cultured overnight at 37° C. to adjust the OD to 1.2 (equivalent to $1*10${circumflex over ( )}9 CFU/mL), and 200 μL/individual (equivalent to $2*10${circumflex over ( )}8 CFU/individual) of bacterial solution was administered into the stomach of mice using a sonde. Regarding the CFU count for Kp-ESBL, DHL medium containing 30 mg/L ampicillin and 30 mg/L spectinomycin was used as a selective medium and cultured overnight at 37° C. under aerobic conditions for calculation.

[0206] As a result, as shown in the lower part (graph) of FIG. **23**, F37mix and K68mix showed the same Kp-ESBL elimination ability as feces F.

Example 15

[0207] As shown in the upper part of FIGS. **24** and **25**, *Campylobacter jejuni* 81-176 (ATCC BAA2151) was administered to germ-free mice, and one week later, mixed F37mix, K68mix, I42mix, or feces F was administered.

[0208] The bacterial solution of *Campylobacter jejuni* was placed in a TS liquid medium, placed in an anaerobic jar together with a slightly aerobic AnaeroPack, and cultured at 42° C. for 48 hours, and the bacterial solution was administered into the stomachs of the mice using a sonde.

[0209] For *Campylobacter jejuni*, CFU and qPCR were used to show the bacterial amount.

[0210] CHROMagar *Campylobacter* was used for CFU counting, which was placed in an anaerobic jar together with a slightly aerobic AnaeroPack and cultured at 42° C. for 48 hours. FIG. **24** shows the obtained results.

[0211] The qPCR measurement was performed according to the following procedure.

[0212] LightCycler (registered trademark) 480II (Roche; 05015243001) and Thunderbird (registered trademark) SYBR (registered trademark) qPCR Mix (TOYOBO; QPS-201X5) were used to amplify and quantify with *Campylobacter jejuni* genome-specific primers and universal bacterial primers, and the calculated DNA concentration ratio was used as the abundance ratio of *Campylobacter jejuni*. FIG. **25** shows the obtained results.

[0213] Note that the primer sets set forth in SEQ ID NOS: 220 and 221 were used as *Campylobacter jejuni* genome-specific primers for qPCR, and the primer sets set forth in SEQ ID NOS: 222 and 223 were used as universal bacterial primers.

[0214] In addition, the bacterial genome was extracted by the following steps.

[0215] A PBS solution containing EDTA and glycerol (final concentration of EDTA: 10 mM, and final concentration of glycerol: 20% by volume) was added 5 times by weight to 10 mg of mouse feces, and the mixture was crushed and suspended with vigorous shaking and stirring. Added to 100 μL of sample solution was 800 μL of 10 mM Tris/10 mM EDTA buffer solution (pH 8.0, also referred to as "TE10" hereinafter) having 15 mg of lysozyme (manufactured by Sigma-Aldrich, Lysozyme from chicken egg white; L4919) and 5 μL of RNase (manufactured by Thermo Fisher Scientific, PureLink RNase A (20 mg/mL); 12091-021) dissolved therein, and the mixture was shaken at 37° C. for 1 hour. Subsequently, 2,000 U of Achromopeptidase (registered trademark) (Wako; 015-09951) was added, and the mixture was shaken at 37° C. for 30 minutes for lysis. Then, 50 μL of a 20% SDS TE10 solution and 50 μL of a TE10 solution having Proteinase K (Roche, Proteinase K, recombinant, PCR Grade; 0311585.101) at a final concentration of 20 mg/ml dissolved therein were added, and the mixture was shaken at 55° C. for 60 minutes. Next, DNA was obtained from 400 μL of the solution using Maxwell (registered trademark) RSC Cultured Cells DNA Kit (Promega).

[0216] As shown in FIGS. **24** and **25**, for *Campylobacter jejuni*, all the mixed bacteria had a good bacterial elimination ability equivalent to that of feces F.

Example 16

[0217] As shown in the upper part of FIG. **26**, *Clostridium difficile* (St. 630) was administered to germ-free mice, and one week later, mixed F37mix, K68mix, I42mix, K47mix, or feces F was administered. Note that K47mix denotes 47 strains isolated from the fecal sample derived from #K, and is duplicated with the 68 bacterial strains (K1 to K46 shown in Tables 1 and 2) except for 1 bacterial strain.

[0218] The bacterial solution of *C. difficle* was subjected to spore formation, adjusted to around 1×10 {circumflex over (   )}5 cells, and administered into the stomachs of the mice using a sonde. The spore formation was performed by culturing in Clospore medium for 8 days in an anaerobic chamber at 37° C. and washing the medium with PBS, followed by treatment with sonication, addition of Lysoizyme and trypsin, and treatment at 45° C. for 6 hours and then at 70° C. for 10 minutes.

[0219] For *C. difficle*, qPCR was used to quantify the bacterial amount. As the primers for qPCR, the primer set shown in SEQ ID NOs: 224 and 225 was used.

[0220] As a result, as shown in the lower part of FIG. **26**, a high elimination ability was observed in K68mix and K47mix for *C. difficile.*

INDUSTRIAL APPLICABILITY

[0221] As described above, by suppressing the colonization of drug-resistant bacteria and pro-inflammatory bacteria in the intestinal tract, the present invention makes it possible to treat, ameliorate, or prevent diseases caused by these bacteria. Therefore, the present invention is

extremely useful in the development, treatment, amelioration, prevention, and the like of pharmaceutical products relating to infectious diseases caused by drug-resistant bacteria or pro-inflammatory bacteria.

Claims

1. An antibacterial composition against drug-resistant bacteria or pro-inflammatory bacteria, said composition comprising active ingredients, wherein the active ingredients are: (1) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 105 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 105, (2) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 69 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 69, (3) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 80 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 80, (4) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 85 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 85, (5) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 86 or a nucleotide sequence having ai least 90% identity with nucleotide sequence of SEQ ID NO: 86, (6) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 87 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 87, (7) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 88 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 88, (8) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 89 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 89, (9) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 90 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 90, (10) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 91 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 91, (11) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 92 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 92, (12) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 94 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 94, (13) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 96 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 96, (14) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 98 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 98, (15) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 99 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 99, (16) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 100 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 100, (17) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 101 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 101, and (18) a bacterium having a DNA composed of the nucleotide sequence of SEQ ID NO: 103 or a nucleotide sequence having at least 90% identity with the nucleotide sequence of SEQ ID NO: 103; and wherein said composition farther comprises a medium and/or a buffer.
2. The antibacterial composition according to claim 1, which is a pharmaceutical composition.