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(54) Title: COMPOSITIONS AND METHODS FOR TREATING ANTIBIOTIC RESISTANCE

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The present invention relates to compositions and methods for reducing the carriage of antibiotic resistance genes.

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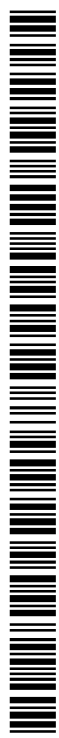
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(57) Abstract: The present invention relates to compositions and methods for reducing the carriage of antibiotic resistance genes.



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COMPOSITIONS AND METHODS FOR TREATING ANTIBIOTIC RESISTANCE

FIELD OF THE INVENTION

5 The invention relates to preventing and treating antibiotic resistance.

BACKGROUND

According to the World Health Organization (WHO), bacterial “[a]ntibiotic resistance is one of the biggest threats to global health, food security, and development today”
10 (who.int/mediacentre/factsheets/antibiotic-resistance/en). Antibiotic resistance can occur naturally in bacteria but is greatly exacerbated by increasing use and misuse of antibiotics. Adding to the problem is the diversity of antibiotic resistance mechanisms; a variety of disparate genes can confer resistance, and unrelated bacteria can horizontally transmit antibiotic resistance genes. The WHO has also stated “[w]hile there are some new antibiotics in development, none of them are expected to be effective
15 against the most dangerous forms of antibiotic-resistant bacteria (*ibid*).”

Accordingly, it is imperative that new methods of reducing antibiotic resistance be developed and made available.

SUMMARY

20 The invention relates to the discovery that bacterial compositions can be used to reduce the abundance of antibiotic resistance genes in the gastrointestinal (GI) microbiome of a subject.

The invention provides compositions including a therapeutically effective amount of a microbiome composition comprising spore-former bacteria for use in reducing the abundance of at least one antibiotic-resistance gene in the microbiome of a subject.

25 The invention also provides compositions including a therapeutically effective amount of a microbiome composition comprising *Firmicutes* derived from one or more healthy human subjects for use in treating a subject at risk for or diagnosed with an undesirable level or population of drug-resistant bacteria.

The invention further provides ROAR compositions for use reducing the abundance of
30 antibiotic-resistant bacteria in the microbiome of a subject.

The invention additionally provides ROAR compositions for use in disrupting a transmission cycle of antibiotic resistance genes, by administration to at least two individuals with a high likelihood of direct or indirect contact, including optionally contact with a bodily fluid or waste.

In addition, the invention provides methods of reducing the abundance of at least one antibiotic-resistance gene in the microbiome of a subject, the methods including administering a therapeutically effective amount of a microbiome composition containing spore-former bacteria.

5 The invention also provides methods of treating a subject at risk for or diagnosed with an undesirable level or population of drug-resistant bacteria, the methods including administering a therapeutically effective amount of a microbiome composition containing *Firmicutes* derived from one or more healthy human subjects.

10 The invention further provides methods of reducing the abundance of antibiotic-resistant bacteria in the microbiome of a subject, the methods including administering a therapeutically effective amount of a ROAR composition.

The invention additionally provides methods of disrupting a transmission cycle of antibiotic resistance genes, the methods including administering a ROAR composition to at least two individuals with a high likelihood of direct or indirect contact including contact with a bodily fluid or waste.

Various embodiments of any of the methods noted above are as set forth below.

15 In some embodiments, the antibiotic-resistance gene(s) is selected from those listed in Table 1.

In some embodiments, the abundance of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more antibiotic resistance genes of Table 1 is reduced.

In some embodiments, the drug or antibiotic is from a drug class listed in Table 2.

20 In some embodiments, the methods further include, or the compositions for use further result in, reducing the abundance of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more antibiotic resistant genes or drug classes.

In some embodiments, the composition includes one or more species listed in Table 3. In some embodiments, the species listed in Table 3 is not also listed in Table 4. The composition may be administered by a method described above or elsewhere herein.

25 In some embodiments, the composition includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more species listed in Table 3. In some embodiments, the species listed in Table 3 is not also listed in Table 4. The composition may be administered by a method described above or elsewhere herein.

30 In some embodiments, the species include a 16S rDNA, V4, and/or V6 sequence listed in Table 5, or a sequence having at least 95% identity thereto. The species may be administered by a method described above or elsewhere herein.

In some embodiments, the composition does not include one or more species of Table 4. In some embodiments, the species listed in Table 4 that is not included is not listed Table 3. The composition may be administered by a method described above or elsewhere herein.

In some embodiments, the species does not include a 16S rDNA, V4, and/or V6 sequence listed in Table 6, or a sequence having at least 95% identity thereto. The species may be administered by a method described above or elsewhere herein.

In some embodiments the bacteria in the composition are in the form of spores.

5 In some embodiments, the bacteria in the composition are cultured.

In some embodiments, the composition includes *Firmicutes* and one or more species of *Bacteroides*.

10 In some embodiments, the subject has a reduced abundance of one, two, or three bacteria selected from the group consisting of quinolone resistant, beta-lactam-resistant, and carbapenem-resistant bacteria.

In some embodiments, the subject has been unresponsive to antibiotic treatment.

15 In some embodiments, the subject has or is at risk of developing *C. difficile* infection or disease, or a colitis wherein, in some embodiments, the colitis is optionally Crohn's disease or ulcerative colitis (e.g., mild to moderate ulcerative colitis). In some embodiments, the subject does not have (or is not risk of developing) *C. difficile* infection or disease, or a colitis (such as Crohn's disease or ulcerative colitis, e.g., mild to moderate ulcerative colitis).

The invention also provides compositions including bacterial species associated with a decrease in the abundance of at least one antibiotic resistance gene in a subject.

In some embodiments, the antibiotic-resistance gene(s) is selected from those listed in Table 1.

20 In some embodiments, the abundance of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more antibiotic resistance genes of Table 1 is decreased.

In some embodiments, the antibiotic is from a class listed in Table 2.

In some embodiments, the composition includes one or more species listed in Table 3. In some embodiments, the species listed in Table 3 is not also listed in Table 4.

25 In some embodiments, the composition includes 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more species listed in Table 3. In some embodiments, the species listed in Table 3 is not also listed in Table 4.

In some embodiments, the species include a 16S rDNA, V4, and/or V6 sequence listed in Table 5, or a sequence having at least 95% identity thereto.

30 In some embodiments, the composition does not include one or more species of Table 4. In some embodiments, the species of Table 4 that is not included is not listed in Table 3.

In some embodiments, the species do not include a 16S rDNA, V4, and/or V6 sequence listed in Table 6, or a sequence having at least 95% identity thereto.

In some embodiments, the composition includes one or more species of *Firmicutes*.

35 In some embodiments, the composition includes one or more species of *Bacteroides*.

In some embodiments, the bacteria in the composition are in the form of spores.

In some embodiments, the bacteria in the composition are obtained from a spore fraction derived from feces of a healthy subject.

In some embodiments, the composition includes one or more species of *Firmicutes*, and optionally one or more species of *Bacteroides*, as the sole bacterial species in the composition.

In some embodiments, the bacteria in the composition are cultured.

The invention further provides compositions including bacteria for use in decreasing the abundance of at least one antibiotic resistance gene in a subject to whom the composition is administered.

In some embodiments, the bacteria in the composition are obtained from a spore fraction derived from feces of a healthy subject.

In some embodiments, the bacteria are selected for being associated with a decrease in the abundance of one or more antibiotic resistance genes in a subject.

In some embodiments, the antibiotic resistance gene(s) is selected from Table 1.

In some embodiments, the composition includes at least one species selected from Table 3.

In some embodiments, the composition does not include a species of Table 4.

The invention also provides methods for identifying a bacterial composition useful for decreasing the abundance of at least one antibiotic resistance gene in a subject to whom the composition is administered, the methods including screening the composition for the presence of one or more bacterial species of Table 3, wherein detection of one or more species of Table 3 in the bacterial composition indicates the identification of a composition that can be used to decrease the abundance of at least one antibiotic resistance gene. In some embodiments, the species of Table 3 is not also listed in Table 4.

In some embodiments, the screening includes detection of one or more 16S rDNA, V4, and/or V6 sequences of Table 5 in the composition, or a sequence having at least 95% identity thereto.

In some embodiments, the methods further include screening for the presence of one or more bacterial species of Table 4. In some embodiments, the species screened for from Table 4 is also not listed in Table 3.

In some embodiments, the screening includes detection of one or more 16S rDNA, V4, and/or V6 sequences of Table 6 in the composition, or a sequence having at least 95% identity thereto.

The invention also includes methods for screening a potential donor of feces for use in therapeutic methods, the methods including testing a feces sample from the potential donor for the presence of a bacterial species from Table 3 or Table 4, wherein detection of one or more species from Table 3 indicates that the potential donor may proceed to become a donor, while detection of one or more species from Table 4 indicates that the potential donor should not become a donor. In some

embodiments, the species from Table 3 tested for is not listed in Table 4. In some embodiments, the species from Table 4 tested for is not listed in Table 3.

The invention further includes methods for determining whether a subject could benefit from treatment with a ROAR composition, the methods including determining whether a sample from the subject includes one or more species of bacteria from Table 4, wherein detection of one or more species from Table 4 indicates that the subject may benefit from treatment with a ROAR composition. In some embodiments, the species from Table 3 tested for is not listed in Table 4. In some embodiments, the species from Table 4 tested for is not listed in Table 3.

The invention further provides compositions (e.g., the compositions described above and elsewhere herein), wherein the bacteria in the compositions demonstrate the ability to decrease the abundance of one or more antibiotic resistance gene(s) in an animal model of antibiotic resistance. In some embodiments, the animal model is a mouse treated with one or more antibiotics and then colonized with one or more bacteria harboring one or more antibiotic resistance genes. In some embodiments, the mice are colonized with vancomycin resistant bacteria or vancomycin and carbapenem resistant bacteria.

The invention also includes use of the compositions described herein for the purposes specified in the methods described herein, as well as the use of these compositions for the preparation of medicaments for these uses.

The entire disclosure of each patent document and scientific article referred to herein, and those patent documents and scientific articles cited thereby, is expressly incorporated by reference herein for all purposes.

Additional features and advantages of the invention are more particularly described below.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph depicting the results of an experiment detecting the reduction of genes associated with fluoroquinolone resistance in human subjects 7, 28, and 56 days after dosing with a human commensal spore composition. White = subjects who received placebo, medium gray = all subjects treated with the spore composition, and dark gray = results from the subset of subjects with a significant engraftment signal.

Figure 2 is a graph depicting the results of an experiment detecting the reduction of genes associated with beta-lactam antibiotic resistance in human subjects 7, 28, and 56 days after dosing with a human commensal spore composition. White = subjects who received placebo, medium gray = all subjects treated with the spore composition, dark gray = results from the subset of subjects with a significant engraftment signal.

Figure 3 is a graph showing the abundance of antibiotic resistance genes per time point (x-axis), faceted by cohort (A: placebo, B: spore prepared from the stool of healthy, screened humans, weekly, D: vancomycin, spore preparation weekly, and C: vancomycin, spore preparation daily).

Figure 4 is a graph showing that in subjects with rapid, early engraftment with spore preparation in Arm D (Vancomycin, spore preparation Weekly), antibiotic resistance genes decreased more rapidly as compared to subjects who did not exhibit rapid, early engraftment. Visit 4 (day 8) is immediately following cessation of antibiotics, and Visit 4 (day 11) is the first spore preparation time point.

DETAILED DESCRIPTION

Antibiotic resistance (referred to herein as “AbxR”) is a growing global public health issue. Resistance to all classes of antibiotics has been reported in all parts of the world (2014, *Antimicrobial Resistance, Global Report on Surveillance, Summary*, WHO) and development of conventional antibiotics has been relatively slow compared to the demand. For example, no new classes of antibiotics have been commercialized since the discovery of the lipopeptide antibiotic daptomycin in the 1980s. Furthermore, antibiotic stewardship programs that limit antibiotic use do not immediately reverse the trend of increasing resistance.

Applicants have discovered that treating a subject with a composition comprising bacteria, for example, spore-former bacteria derived from a healthy human, can reduce the abundance of AbxR genes (collectively, the “gut resistome”) in a human subject. Such compositions are referred to herein as reduction of antibiotic resistance compositions (ROAR compositions).

Furthermore, such treatment can reduce the abundance of AbxR genes associated with resistance to multiple classes of antibiotics, e.g., β -lactams (including carbapenems), quinolones, glycopeptides (e.g., vancomycin), and tetracyclines. In addition, it was found that non-pathogenic commensals can harbor antibiotic resistance genes, and treatment results in a decrease in the abundance of antibiotic resistance genes in non-pathogenic commensal organisms. Without committing to any particular theory, these data support a method of decreasing the presence of antibiotic resistance genes by, at least in part, a mechanism of decreasing horizontal transfer of antibiotic resistance genes from non-pathogenic commensal organisms to pathogenic bacteria. Such a mechanism could be related at least in part to decreasing the abundance of bacteria harboring antibiotic resistance genes by out-competing these bacteria for nutrients; decreasing the abundance of bacteria harboring antibiotic resistance genes by inducing a response in the host that limits the growth of potential pathogens harboring AbxR genes; decreasing the abundance of bacteria harboring antibiotic resistance genes by introducing bacteria that are directly antagonistic to potential pathogens harboring

AbxR genes; reducing opportunistic pathogens, and/or decreasing the potential for the spread of antibiotic resistance genes between antibiotic resistant and susceptible organisms. In some embodiments, the decrease in the presence of antibiotic resistance genes occurs by reducing colonization in the host of microbes that harbor AbxR genes.

In some embodiments, a ROAR composition is used to break or prevent an antibiotic resistance transmission cycle. In such methods, a ROAR composition can be administered to a subject entering an environment in which there is a risk of an antibiotic resistance cycle or an environment in which the existence of an antibiotic resistance cycle has been identified, e.g., an environment in which antibiotic resistance has been identified as spreading or in which there is a risk of such spreading. Examples of such environments include hospitals, intensive care units, nursing homes, rehabilitation centers, group homes, and the like. In some embodiments, the dosing in such an environment is chronic. In some embodiments, a subject is treated to prevent or reduce colonization by one or more AbxR genes. In a subject that harbors one or more AbxR genes, a composition can be administered, thereby reducing the risk of AbxR infection in that subject and reducing the risk that subject will transmit AbxR genes after treatment of *C. difficile* disease, as such subjects may be susceptible to infection with or harbor antibiotic resistant bacteria. In some embodiments, a composition is administered to workers in environments in which there is a particular risk associated with spreading AbxR genes.

In some environments, “selective decontamination” of patients has been recommended, for example, in hospitals (particularly intensive care units) and nursing homes (for example, see Silvestri and van Saene, “Selective decontamination of the digestive tract: an update of the evidence,” HSR Proc. Intensive Care Cardiovasc. Anesth. 4:21-29, 2012; Oostdijk et al., “Selective decontamination in European intensive care patients,” Intensive Care Med. 38:533-538, 2012). A ROAR composition can be a useful alternative or supplement to such selective decontamination, adding to the armamentarium of methods available to decrease the presence of antibiotic resistance genes in these and other settings.

ROAR Compositions

ROAR compositions generally comprise *Firmicutes*. In some embodiments, the *Firmicutes* are capable of forming spores (spore-formers). Bacteria in a composition can be in the form of spores, mixed vegetative and spore forms, or vegetative form. In some cases, a composition consists of *Firmicutes*. In some embodiments, a ROAR composition comprises one or more species of *Bacteroidetes*. In some embodiments, a ROAR composition consists of at least one species from each of *Firmicutes* and *Bacteroidetes*. Compositions comprising or consisting of *Firmicutes* (or *Firmicutes* and *Bacteroidetes*) as the sole bacterial species of the compositions are not naturally occurring.

As a non-limiting example, a ROAR composition can be prepared from bacteria that are isolated from stool, e.g., human stool. In general, the stool is from a healthy animal (e.g., a human) of

the same species as the animal to be treated. Bacteria can be prepared, for example, in a method using an amount of ethanol suitable to kill any non-spore form bacteria present in the stool. An example of such a preparation can be found in, for example, WO 2014/121302.

In some embodiments, a ROAR composition comprises or consists of selected bacterial species associated with a decrease in the abundance of antibiotic resistance genes in a subject (see, e.g., Table 3, below). Such compositions are referred to herein as “designed Abx compositions.” The number of species (e.g., from Table 3) in a designed composition can be, for example, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more. In some embodiments the number of species is at least one and less than or equal to 50, for example, less than 45, less than 40, less than 35, less than 30, less than 25, less than 20, less than 15, less than 10, or less than 5. In some embodiments, the species selected from Table 3 do not include species that are also listed in Table 4.

Individual species in a designed ROAR composition may be cultured separately and then combined to create the composition. In some embodiments, a ROAR composition is produced by co-culturing all or subsets of the composition and then combining the components that were not co-cultured to create the composition. In some embodiments, a ROAR composition is produced by culturing a spore fraction derived from feces of a healthy human. The resulting culture can be used as a ROAR composition.

In some embodiments, the species selected for inclusion in a ROAR composition are associated with a decrease in the abundance of a particular class of antibiotic resistance genes, e.g., genes associated with resistance to quinolones, beta-lactams, and/or carbapenems (also see Table 2). In specific examples of, e.g., the compositions described above, bacterial species in a ROAR composition (e.g., designed Abx compositions) are selected from Table 2, and optionally may be identified by analysis of 16S rDNA sequences (or fragments thereof, e.g., a fragment comprising the 16S rDNA V4 and/or V6 regions; also see below and Table 5 for exemplary sequences).

In some embodiments, a species useful in a ROAR composition is a species having a full-length 16S rDNA with at least 95% sequence identity (“identity”) (e.g., at least 96%, 97%, 98%, or 99%, or 100%) to the 16S rDNA of a reference species, e.g., a species identified in Table 3 (see, e.g., Table 5 for sequence information). In some embodiments, a species useful in a ROAR composition is a species having a full-length 16S rDNA with at least 97% sequence identity (“identity”) to the 16S rDNA of a reference species, e.g., a species in Table 3 (see, e.g., Table 5 for sequence information). In some embodiments, a useful species has a V4 and/or V6 region 16S rDNA sequence having at least 95% identity (e.g., at least 96%, 97%, 98%, or 99%, or 100%) to a V4 and/or V6 region of 16S rDNA sequence of a reference species, e.g., a species identified in Table 3 (see, e.g., Table 5 for sequence information). In some embodiments, a useful species has a V4 and/or V6 region 16S rDNA sequence having at least 97% identity to a V4 and/or V6 region of 16S rDNA sequence of a reference species,

e.g., a species identified in Table 3 (see, e.g., Table 5 for sequence information). In some embodiments, a useful species has a genomic sequence having at least 95% identity (e.g., at least 96%, 97%, 98%, or 99%, or 100%) to the full-length genomic DNA of a reference species, e.g., a species identified in Table 3. In some embodiments a useful species has a genomic sequence having at least 97% identity to the full-length genomic sequence of a reference species, e.g., a species identified in Table 3. In the event that a sequence is not provided herein, e.g., a 16S rDNA, V4, or V6 sequence, methods are well known in the art for identifying such sequences. Table 5 provides non-limiting examples of full-length 16S rDNA sequences that can be used as reference sequences. In general, identity or percent identity with a reference species means identity or percent identity with at least one 16S rDNA sequence (or fragment; e.g., V4 and/or V6) found in an organism.

In some cases, strains of bacterial species useful in the invention, e.g., species disclosed herein, can be obtained from a public biological resource center such as the ATCC (atcc.org), the DSMZ (dsmz.de), or the Riken BioResource Center (en.brc.riken.jp). 16s rDNA sequences useful for identifying species or other aspects of the invention can be obtained from public databases, e.g., the Human Microbiome Project (HMP) web site or GenBank.

Methods of determining sequence identity are known in the art and examples are provided *infra*.

In some embodiments, species excluded from ROAR compositions are species that are positively correlated with the presence of a particular class of antibiotic resistance genes (see, e.g., Table 4). These species can be identified using methods such as those described above (also see Table 6 for corresponding sequence information).

It is to be understood that “consisting of” in these examples refers to bacteria types that are present in a composition. A bacterial formulation “consisting of” listed species may thus contain additional non-bacterial materials such as one or more excipients (including, for example, one or more capsules), an aqueous or non-aqueous medium (e.g., glycerol, polyethylene glycol, cocoa butter, water, and/or a buffer), and/or one or more prebiotics or small molecule drugs.

Species/naming information

Names and classification of bacteria are subject to changes that may not be reflected in the literature. For convenience, alternate names for some bacterial species may be provided herein but are not intended to be a comprehensive set of alternative names. In some embodiments, species are identified by sequence identity of all or a portion (e.g., V4 or V6 regions) of a 16S rDNA sequence, e.g., at least 90%, 93% 95%, 96%, 97%, 98%, 99%, or 100% identity.

Determination of identity

Clades, operational taxonomic units (OTUs), species, and strains are, in some embodiments, identified by their 16S rDNA sequence. The relatedness of clades, OTUs, species, and strains can be determined by the percent identity between clades, OTUs, species, or strains. Percent identity between a reference and query sequence can be determined using methods known in the art. Non-limiting examples of methods for such determinations are provided below. As used herein, the relatedness between two nucleotide sequences is described by the parameter “identity.”

In one embodiment, the degree of sequence identity between a query sequence and a reference sequence is determined by (1) aligning the two sequences by any suitable alignment program using the default scoring matrix and default gap penalty, (2) identifying the number of exact matches, where an exact match is where the alignment program has identified an identical nucleotide in the two aligned sequences on a given position in the alignment, and (3) dividing the number of exact matches with the length of the reference sequence.

In another embodiment, the degree of sequence identity between a query sequence and a reference sequence is determined by (1) aligning the two sequences by any suitable alignment program using the default scoring matrix and default gap penalty, (2) identifying the number of exact matches, where an exact match is where the alignment program has identified an identical nucleotide in the two aligned sequences on a given position in the alignment, and (3) dividing the number of exact matches with the length of the longest of the two sequences.

In another embodiment, the degree of sequence identity between the query sequence and the reference sequence is determined by (1) aligning the two sequences by any suitable alignment program using the default scoring matrix and default gap penalty, (2) identifying the number of exact matches, where an exact match is where the alignment program has identified an identical amino acid or nucleotide in the two aligned sequences on a given position in the alignment, and (3) dividing the number of exact matches with the “alignment length,” where the alignment length is the length of the entire alignment including gaps and overhanging parts of the sequences.

Sequence identity comparisons are carried out, generally, with the aid of a sequence comparison program. These commercially or publicly available computer programs use complex comparison algorithms to align two or more sequences that best reflect the evolutionary events that might have led to the difference(s) between the two or more sequences. Therefore, these algorithms operate with a scoring system rewarding alignment of identical or similar amino acids and penalizing the insertion of gaps, gap extensions, and alignment of non-similar amino acids. The scoring system of the comparison algorithms include:

- i) assignment of a penalty score each time a gap is inserted (gap penalty score),

ii) assignment of a penalty score each time an existing gap is extended with an extra position (extension penalty score),

iii) assignment of high scores upon alignment of identical amino acids, and

iv) assignment of variable scores upon alignment of non-identical amino acids.

5 In general, the default values of the alignment program are used for sequence comparisons. Suitable computer programs useful for determining identity include, for example, BLAST (blast.ncbi.nlm.nih.gov).

In an embodiment of the present invention, the alignment program optimizes the alignment over the full-length of selected sequences, e.g., full-length, V4, or V6 16S rDNA sequence. For
10 example, the global alignment program is based on the Needleman-Wunsch algorithm (Needleman and Wunsch, J. Mol. Biol. 48: 443-453, 1970). Non-limiting examples of such programs are EMBOSS Needle and EMBOSS Stretcher programs, available at ebi.ac.uk/Tools/psa/.

In one embodiment, the sequences are aligned by a global alignment program and the sequence identity is calculated by identifying the number of exact matches identified by the program divided by
15 the “alignment length,” where the alignment length is the length of the entire alignment including gaps and overhanging parts of the sequences. In a further embodiment, the global alignment program uses the Needleman-Wunsch algorithm and the sequence identity is calculated by identifying the number of exact matches identified by the program divided by the “alignment length,” where the alignment length is the length of the entire alignment including gaps and overhanging parts of the sequences.

20 In yet a further embodiment, the global alignment program is selected from the group consisting of EMBOSS Needle and EMBOSS stretcher and the sequence identity is calculated by identifying the number of exact matches identified by the program divided by the “alignment length,” where the alignment length is the length of the entire alignment including gaps and overhanging parts of the sequences.

25 Once the software has produced an alignment, it is possible to calculate percent (%) similarity and percent sequence identity.

Methods of testing compositions for AbxR activity or the presence of AbxR genes

A ROAR composition can be tested for the ability to reduce the abundance of antibiotic
30 resistance genes in the GI tract of an animal using *in vitro* and/or *in vivo* models known in the art (for example, see Schjørring and Krogfelt, Int. J. Microbiol. 2011:312956, 2011; Marra, “Animal models in drug development for MRSA,” Meth. Mol. Biol. 1085:333-345, 2014). Such models can be adapted for use in testing candidate ROAR compositions. Additional examples of suitable models are described in the Examples. In addition, a ROAR composition can be tested for the ability to reduce the
35 abundance of AbxR genes in human subjects enrolled in clinical trials.

Subjects

In some embodiments, a subject suitable for treatment with a composition disclosed herein (a ROAR composition) is an animal, e.g., a mammal such as a human, dog, cat, bovine, ovine, caprine, equine, or other farm, domestic, or zoo animal at risk for, or diagnosed with, having an undesirable level of AbxR microorganisms, e.g., in at least a portion of the gastrointestinal (GI) tract. An undesirable level of AbxR organisms can be identified as a level significantly greater than that in a healthy population, for example, in the context of treating humans, the healthy population documented in the Human Microbiome Project database. Such organisms can be detected by, for example, culturing a stool or other gastrointestinal sample from the subject and using known methods of testing for the presence of antibiotic resistance. The methods include, for example, dilution methods (broth and agar dilution methods), disk-diffusion methods, E-test, automated methods, mechanism-specific tests such as beta-lactamase detection test and chromogenic cephalosporin tests, and genotypic methods such as PCR and DNA hybridization methods. Such methods also include the identification and quantification of antibiotic resistance associated genes by whole metagenomics shotgun (WMS) sequencing. In some embodiments, an undesirable level of AbxR organisms or genes can be identified using molecular methods that identify the presence or absence of genes, gene cassettes, or genetic markers associated with the phenotype of AbxR. Some examples of such molecular methods include but are not limited to DNA sequencing, transcriptional profiling, and methods that utilize a molecular probe.

In some embodiments, a subject suitable for treatment with a ROAR composition is or has been unresponsive to treatment with one or more antibiotics and the presence of an antibiotic resistant microbial population is inferred.

In some embodiments, a subject suitable for treatment with a ROAR composition has or is at risk of developing *C. difficile* infection or disease. In some embodiments, a subject suitable for treatment with a ROAR composition has or is at risk of developing a colitis (e.g., Crohn's disease or ulcerative colitis (e.g., mild to moderate ulcerative colitis)).

Advantages

Use of conventional antibiotics, in addition to the potential for the existence or development of antibiotic resistance by a subject's microbiome, can cause serious side effects including, for example, GI effects such as dysbiosis, overgrowth of pathogens, nervous system damage, and allergic reactions. A ROAR composition typically comprises commensal bacteria and has limited side effects, e.g., is well-tolerated by subjects receiving the treatment. A ROAR composition can be administered repeatedly over time, as needed, and may reduce the risk of developing antibiotic resistance to a large

group of antibiotics, e.g., by reducing the prevalence of antibiotic resistance genes in a treated individual. In general, a ROAR composition can modulate transmission cycles in which bacteria transmit antibiotic resistance genes. This feature is useful in, for example, health care settings in which the use of the compositions decreases the risk of fragile patients being exposed to or developing antibiotic resistance, both individually and as a patient population.

In some embodiments, an additional advantage of an ROAR composition that administration of the composition may improve the integrity of the epithelial and mucosal barriers, for example, by reducing the amount of local inflammation, and reducing the risk of translocation and infection by GI local microbes.

Definitions

“Augmentation” of a type of bacterium, e.g., a species, is an effect of treatment with a composition of the invention that is characterized by post-treatment detection of an increased abundance of a species not present in the composition by a nonparametric test of abundance.

“Engraftment” of a type of bacterium, e.g., a species, is an effect of treatment with a composition of the invention that is characterized by post-treatment detection of a species from the administered composition, which is not detected in the treated subject pretreatment. Methods of detection are known in the art. In one example, the method is PCR detection of a 16S rDNA sequence using standard parameters for PCR.

“Operational taxonomic unit,” “OTU” (or plural, “OTUs”) refers to a terminal leaf in a phylogenetic tree and is defined by a nucleic acid sequence, e.g., the entire genome, or a specific genetic sequence, and all sequences that share sequence identity to this nucleic acid sequence at the level of species. In some embodiments the specific genetic sequence is the 16S sequence or a portion of the 16S sequence, such as a variable region, e.g., a V4 region. In other embodiments, the entire genomes of two entities are sequenced and compared. In another embodiment, select regions such as multilocus sequence tags (MLST), specific genes, or sets of genes may be genetically compared. In 16S embodiments, OTUs that share $\geq 97\%$ average nucleotide identity across an entire 16S or a variable region of a 16S sequence are considered the same OTU (see e.g., Claesson et al., *Nucleic Acids Res.* 38:e200, 2010; Konstantinidis et al., *Philos. Trans. R. Soc. Lond. B. Biol. Sci.* 361:1929-1940, 2006). In embodiments involving the complete genome, MLSTs, specific genes, or sets of genes, OTUs that share $\geq 95\%$ average nucleotide identity are considered the same OTU (see e.g. Achtman and Wagner, *Nat. Rev. Microbiol.* 6:431-440, 2008). OTUs can be distinguished, in some embodiments, by comparing sequences between organisms. Generally, sequences with less than 95% sequence identity are not considered to form part of the same OTU. OTUs may also be characterized by any combination of nucleotide markers or genes, in particular highly conserved genes (e.g., “house-

keeping" genes), or a combination thereof. Such characterization employs, e.g., WGS data or a whole genome sequence. As used herein, the terms "species" and "OTU" are used interchangeably unless otherwise distinguished by context.

A "therapeutically effective amount" of a composition described herein can vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the composition to elicit a desired response in the individual, e.g., amelioration of at least one disorder parameter, or amelioration of at least one symptom of the disorder (and optionally, the effect of any additional agents being administered). A therapeutically effective amount is also one in which any toxic or detrimental effects of the composition are outweighed by the therapeutically beneficial effects. A composition as described herein is generally administered in a therapeutically effective amount.

Formulations

ROAR compositions (e.g., designed Abx compositions) described herein can be prepared and administered using methods known in the art. In general, compositions are formulated for oral, colonoscopic, or nasogastric delivery although any appropriate method can be used.

A ROAR formulation can contain one or more pharmaceutical excipients suitable for the preparation or delivery of such formulations. In some embodiments, the formulation is a liquid formulation. In some embodiments, a formulation comprising a ROAR composition can comprise one or more of surfactants, adjuvants, buffers, antioxidants, tonicity adjusters, thickeners, viscosity modifiers, and the like.

In some embodiments, treatment includes administering a ROAR composition in a formulation that includes a pharmaceutically acceptable carrier. In some embodiments, the excipient includes a capsule or other format suitable for providing the ROAR composition as an oral dosage form. When an excipient serves as a diluent, it can be a solid, semi-solid, or liquid material, which acts as a vehicle, carrier, or medium for the active ingredient. Thus, the formulations can be in the form of tablets, pills, powders, lozenges, sachets, cachets, elixirs, suspensions, emulsions, solutions, syrups, soft or hard capsules, suppositories, or packaged powders.

Some examples of suitable excipients include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose, water, syrup, polyethylene glycol, glycerol, and methyl cellulose. The compositions can be formulated so as to provide quick, sustained, or delayed release of the active ingredient after administration to the patient by employing procedures known in the art.

A ROAR composition can be formulated in a unit dosage form. In general, a dosage comprises about 10^2 to 10^9 viable colony forming units (cfu). In some embodiments, the ROAR compositions

comprises or is composed of spore-former bacteria in spore form. In such cases, the dosage may be determined as “spore CFU” or “sCFU”, which quantify the number of viable spores by germination and growth, typically on plates.

The term “unit dosage forms” refers to physically discrete units suitable as unitary dosages for human subjects and/or other mammals, each unit containing a predetermined quantity of active material calculated to produce a desired therapeutic effect, in association with a suitable pharmaceutical excipient. A dosage may be administered in multiple delivery vehicles, e.g., multiple pills or capsules.

The amount and frequency of administering a ROAR composition to a patient will vary depending upon what is being administered, the purpose of the administration, such as prophylaxis or therapy, the state of the patient, the manner of administration, and the like. In therapeutic applications, compositions can be administered to a patient already suffering from a disease in an amount sufficient to cure or at least partially arrest one or more symptoms of the disease or its complications. Effective doses will depend on the disease or condition being treated, as well as by the judgment of the attending clinician, depending upon factors such as the severity of the disease, the age, weight, and general condition of the patient, and the like.

The dosage can refer, for example, to the total number of cfus of each individual species or strains, or can refer to the total number of microorganisms in the dose. It is understood in the art that determining the number of organisms in a dosage is not exact and can depend on the method used to determine the number of organisms present. For example, the number of spores in a composition may be determined using a dipicolinic acid assay. In some cases, the number of organisms may be determined using a culture assay. When spores are present, the ability of assays relying on culture methods can depend on efficient germination of spores. Quantitative nucleic acid-based methods can depend on whether the nucleic acids from non-viable microorganisms are sufficiently reduced or eliminated. Effective doses can be extrapolated from dose-response curves derived from in vitro or animal model test systems.

Screening methods

The invention also provides methods for identifying bacterial compositions that can be used to decrease the abundance of antibiotic resistance genes in subjects. In these methods, candidate compositions are analyzed for the presence of one or more bacterial species of Table 3. Detection of one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) of these species indicates that the composition may be useful in decreasing the abundance of antibiotic resistance genes. Additional testing can be done to further screen candidate compositions. For example, candidate compositions can further be analyzed for the presence of one or more species of

Table 4. In some embodiments, it may be determined that it is preferable to avoid use of compositions identified as including one or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more) species of Table 4.

In other screening methods of the invention, potential donors of feces for use in therapeutic methods (e.g., therapeutic methods of the present invention) are screened for their suitability to serve as donors. In these methods, a fecal sample of a potential donor is screened for the presence of one or more bacterial species associated with decreased antibiotic resistance genes (see, e.g., Table 3; see Table 5 for sequences) and/or one or more bacterial species correlated with the presence of antibiotic resistance genes (see, e.g., Table 4; see Table 6 for sequences). Detection of the former may indicate that the potential donor is suitable to proceed to serve as a donor, while detection of the latter may indicate that the potential donor should not proceed, as can be determined by those of skill in the art.

Additional screening methods include those used to determine whether a subject may benefit from treatment with a ROAR composition, as described herein. In these methods, a sample from the subject (e.g., a fecal sample) is tested for the presence of one or more bacterial species correlated with the presence of antibiotic resistance genes (see, e.g., Tables 4 and 6). Detection of one or more of these species can indicate that the subject may benefit from treatment according to the therapeutic methods of the invention, as can be determined by those of skill in the art.

Detection of species in these methods of the invention can be carried out using methods that are known in the art (see, e.g., above and elsewhere herein). For example, methods including, for example, qPCR, whole metagenomics shotgun sequencing (WMS; see, e.g., below), and/or analysis of 16S rDNA sequences or a portion thereof (e.g., the V4 and/or V6 regions; see, e.g., Table 5) can be performed. Sequences from Table 5 can be detected with respect to the identification of negative correlates (i.e., reduction in antibiotic resistance genes). Sequences from Table 6 can be detected with respect to the identification of positive correlates (i.e., increased abundance of antibiotic resistance genes).

EQUIVALENTS

All technical features can be individually combined in all possible combinations of such features.

The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein.

EXAMPLES

The following non-limiting examples further illustrate embodiments of the inventions described herein.

Example 1: Human studies - *Clostridium difficile*

Human subjects at risk for recurrent *Clostridium difficile* infection (rCDI) were treated with enriched and purified spores prepared from the stool of healthy, screened humans in a randomized, double-blind, placebo-controlled Phase 2 trial (n=89; randomized 2:1; 36 sites). The spores were administered orally in a single dose (of 4 capsules). Dosing was at 10e8 spore equivalents. The GI microbiomes of these subjects, as well as subjects in a Phase 1b open label dose ranging study, were profiled using whole metagenomics shotgun sequencing (WMS). Stool samples were collected before treatment with the spores (after treatment with antibiotics) and after receiving spores or placebo, as applicable.

DNA was extracted from stool samples and used to generate WMS data with the Illumina sequencing platform. WMS data was screened for the presence and abundance of genetic markers of proteins known to confer antibiotics resistance, as catalogued in the Comprehensive Antibiotic Resistance Database (CARD v.1.1.8) (card.mcmaster.ca). Genetic markers associated with these proteins were constructed using ShortBRED (Kaminski et al., PLoS Comput Biol 11(12):e1004557), an informatics tool designed to identify proteins of interest at high specificity within WMS data (Table 1). For each class of antibiotics, the abundance of genetic markers associated with resistance to that class summed to generate normalized-gene abundance values (referred to herein as drug class marker-abundance or DCMA).

For the Phase 2 study, differential prevalence of spore-former species was observed based on WMS analysis. Gene abundance was assessed using the ShortBRED markers (Kaminski et al., supra) for genes associated with antibiotic resistance identified in ARDB (Liu and Pop, 2009, ARDB—Antibiotic Resistance Genes Database. Nuc Acids Res 37(Database issue):D443–447). Data were analyzed for a significant engraftment signal, defined as subjects in whom a significant number of species were identified as being significantly genetically similar to species identified in the administered ROAR composition. Analysis of the data resulted in the discovery that in subjects with a significant engraftment signal for the composition, the abundance of genes associated with resistance to multiple classes of drugs, i.e., quinolone drugs (Fig. 1) and beta-lactam or carbapenem drugs (Fig. 2), was reduced.

In additional analyses, for each subject and drug class (from both the Phase 1b and Phase 2 studies), we calculated the log2 fold change (log2FC) of drug class marker abundance (DCMA) between samples collected at the visit 1 week after receiving placebo or spores, as compared to samples collected one day prior to treatment with placebo or spores. We compared rCDI subjects who

received placebo to rCDI subjects who received spores and in whom significant engraftment was detected. We used the non-parametric Mann-Whitney test to determine if the log₂FC in DCMA was significantly greater in subjects with engraftment of spores as compared to subjects who received placebo (Table 2). False discovery rates (FDR), the expected proportion of incorrectly rejected null hypotheses for a given p-value due to running multiple tests, was calculated using the Benjamini-Hochberg procedure ($p < 0.1$)

The data demonstrate that a ROAR composition can be used to reduce the abundance of antibiotic resistance genes in a subject.

10 *Table 1 – Change in abundance of genes in response to treatment*

CARD accession	p-value	FDR	log ₂ Engrafter - log ₂ Placebo	Gene Symbol
ARO_3000753	1.5E-03	8.5E-02	0.91	abeM
ARO_3000216	2.9E-03	8.5E-02	-1.24	acrB
ARO_3000828	2.5E-03	8.5E-02	-1.38	baeR
ARO_3000830	1.3E-03	8.5E-02	-1.57	cpxA
ARO_3002867	2.7E-03	8.5E-02	1.35	dfrF
ARO_3004039	3.7E-03	8.5E-02	-1.27	Escherichia coli emrE
ARO_3002522	2.4E-04	8.5E-02	0.90	novA
ARO_3000196	1.1E-03	8.5E-02	1.72	tet32
ARO_3002871	2.2E-03	8.5E-02	1.46	tet37
ARO_3000195	7.4E-04	8.5E-02	-1.28	tetB(P)
ARO_3002829	3.0E-03	8.5E-02	0.70	vgaA
ARO_3000499	5.1E-03	9.5E-02	-1.47	acrE
ARO_3002626	6.1E-03	9.5E-02	-0.76	ANT(6)-Ia
ARO_3003838	5.7E-03	9.5E-02	-1.50	gadW
ARO_3002836	5.6E-03	9.5E-02	-0.50	lnuB
ARO_3000263	6.7E-03	9.5E-02	-1.33	marA
ARO_3000793	6.3E-03	9.5E-02	-1.40	mdtB
ARO_3000794	6.7E-03	9.5E-02	-1.59	mdtC
ARO_3000180	4.9E-03	9.5E-02	-0.53	tetA(P)
ARO_3000194	5.1E-03	9.5E-02	1.57	tetW
ARO_3003202	5.4E-03	9.5E-02	-0.30	TLA-1
ARO_3000796	7.4E-03	1.0E-01	-1.65	mdtF
ARO_3000615	8.5E-03	1.1E-01	-1.41	mefA
ARO_3002818	8.3E-03	1.1E-01	-1.24	msrB
ARO_3000024	9.0E-03	1.1E-01	-1.02	patA
ARO_3000829	1.0E-02	1.2E-01	-1.47	baeS
ARO_3000491	1.1E-02	1.2E-01	-1.11	acrD
ARO_3000656	1.3E-02	1.2E-01	-1.17	acrS
ARO_3000074	1.1E-02	1.2E-01	-1.10	emrB

ARO_3000309	1.2E-02	1.2E-01	-1.33	emrD
ARO_3000206	1.5E-02	1.2E-01	-1.15	emrK
ARO_3000254	1.4E-02	1.2E-01	-1.42	emrY
ARO_3000833	1.3E-02	1.2E-01	-1.20	evgS
ARO_3000795	1.3E-02	1.2E-01	-1.31	mdtE
ARO_3003550	1.4E-02	1.2E-01	-1.20	mdtP
ARO_3003950	1.3E-02	1.2E-01	-1.48	msbA
ARO_3003952	1.4E-02	1.2E-01	-1.12	yojI
ARO_3003549	1.5E-02	1.3E-01	-1.14	mdtO
ARO_3002630	1.8E-02	1.3E-01	-0.41	ANT(9)-Ia
ARO_3002634	1.9E-02	1.3E-01	-1.04	APH(2'')-Ie
ARO_3001328	1.9E-02	1.3E-01	-1.02	Escherichia coli mdfA
ARO_3000832	1.8E-02	1.3E-01	-1.18	evgA
ARO_3000676	1.7E-02	1.3E-01	-1.08	H-NS
ARO_3003206	1.8E-02	1.3E-01	-0.40	IscA
ARO_3001329	2.0E-02	1.3E-01	-1.05	mdtG
ARO_3001216	1.8E-02	1.3E-01	-1.12	mdtH
ARO_3003107	1.8E-02	1.3E-01	-0.20	mefB
ARO_3002174	2.0E-02	1.3E-01	-1.21	MIR-9
ARO_3000237	1.9E-02	1.3E-01	-1.11	tolC
ARO_3000502	2.4E-02	1.4E-01	-0.91	acrF
ARO_3002986	2.4E-02	1.4E-01	-1.33	bacA
ARO_3002669	2.9E-02	1.5E-01	-0.90	APH(2'')-Ig
ARO_3002152	2.9E-02	1.5E-01	-1.25	DHA-21
ARO_3000516	2.9E-02	1.5E-01	-1.23	emrR
ARO_3002985	3.2E-02	1.7E-01	-0.84	arnA
ARO_3003057	3.4E-02	1.7E-01	-0.23	smeF
ARO_3000803	3.4E-02	1.7E-01	-0.18	MexE
ARO_3001304	3.5E-02	1.7E-01	-0.49	ErmS
ARO_3002559	3.6E-02	1.7E-01	-1.06	AAC(6')-I _p
ARO_3003017	3.7E-02	1.7E-01	-0.09	dfrA21
ARO_3002804	3.7E-02	1.7E-01	-0.37	FosA2
ARO_3002894	3.7E-02	1.7E-01	-0.06	otrC
ARO_3003576	3.7E-02	1.7E-01	-0.89	PmrC
ARO_3003841	3.8E-02	1.7E-01	-0.84	kdpE
ARO_3003578	4.0E-02	1.8E-01	-0.87	pmrF
ARO_3002597	4.4E-02	2.0E-01	-0.93	AAC(6')-Ie-APH(2'')-Ia
ARO_3002635	4.4E-02	2.0E-01	-1.22	APH(2'')-IIa
ARO_3000205	4.8E-02	2.1E-01	-1.14	tetX
ARO_3000192	4.9E-02	2.1E-01	-0.15	tetS
ARO_3004042	5.0E-02	2.1E-01	-1.14	Enterobacter cloacae acrA
ARO_3003773	5.6E-02	2.3E-01	-0.04	Clostridium perfringens mprF

ARO_3002859	5.6E-02	2.3E-01	0.23	dfrA14
ARO_3003548	5.5E-02	2.3E-01	-0.87	mdtN
ARO_3002688	5.8E-02	2.3E-01	0.21	catS
ARO_3002835	5.8E-02	2.3E-01	-0.21	lnuA
ARO_3000518	6.1E-02	2.3E-01	-0.99	CRP
ARO_3000792	6.2E-02	2.3E-01	-0.60	mdtA
ARO_3000826	6.1E-02	2.3E-01	-0.90	sdiA
ARO_3002671	6.3E-02	2.3E-01	-0.32	cat-TC
ARO_3003112	6.3E-02	2.3E-01	0.11	lsaC
ARO_3002670	6.9E-02	2.5E-01	0.70	cat
ARO_3000508	6.9E-02	2.5E-01	-0.81	gadX
ARO_3000300	7.0E-02	2.5E-01	-0.22	lsaA
ARO_3003699	7.0E-02	2.5E-01	-0.25	mexQ
ARO_3000191	6.8E-02	2.5E-01	-1.21	tetQ
ARO_3000027	7.2E-02	2.5E-01	-0.76	emrA
ARO_3000377	7.5E-02	2.6E-01	-0.25	MexA
ARO_3003704	8.0E-02	2.6E-01	-0.10	mexM
ARO_3003705	8.0E-02	2.6E-01	-0.24	mexN
ARO_3001421	7.8E-02	2.6E-01	0.03	OXA-26
ARO_3002639	8.3E-02	2.6E-01	-0.51	APH(3'')-Ib
ARO_3004072	8.5E-02	2.6E-01	-0.22	OpmB
ARO_3003681	8.5E-02	2.6E-01	-0.22	TriC
ARO_3002932	8.3E-02	2.6E-01	-0.30	vanSB
ARO_3002831	8.4E-02	2.6E-01	-1.15	vgaC
ARO_3000250	8.9E-02	2.7E-01	-0.27	ErmC
ARO_3002964	8.9E-02	2.7E-01	-0.35	vanWB
ARO_3003679	9.0E-02	2.7E-01	-0.13	TriA
ARO_3002950	9.5E-02	2.8E-01	-0.39	vanXB
ARO_3002553	9.8E-02	2.9E-01	-0.02	AAC(6')-Ib
ARO_3001777	9.8E-02	2.9E-01	-0.04	OXA-347
ARO_3001303	1.1E-01	3.0E-01	0.07	ErmO
ARO_3000823	1.1E-01	3.0E-01	-0.98	ramA
ARO_3002933	1.1E-01	3.0E-01	-0.12	vanSC
ARO_3003031	1.1E-01	3.1E-01	-0.34	mexW
ARO_3002679	1.1E-01	3.1E-01	-0.25	catB7
ARO_3000578	1.2E-01	3.4E-01	-0.21	CcrA beta-lactamase
ARO_3002156	1.2E-01	3.4E-01	0.16	FOX-2
ARO_3000811	1.2E-01	3.4E-01	-0.04	mtrD
ARO_3001299	1.2E-01	3.4E-01	0.02	tlrB conferring tylosin resistance
ARO_3002502	1.3E-01	3.4E-01	-0.23	PDC-5
ARO_3000149	1.3E-01	3.5E-01	-0.15	fosA
ARO_3000378	1.4E-01	3.6E-01	-0.29	MexB
ARO_3002923	1.4E-01	3.6E-01	-0.59	vanRD

ARO_3003577	1.4E-01	3.7E-01	-0.65	pmrE
ARO_3003922	1.4E-01	3.7E-01	-0.99	oqxA
ARO_3000806	1.5E-01	3.7E-01	-0.18	mexG
ARO_3002629	1.5E-01	3.9E-01	0.74	ANT(6)-Ib
ARO_3002868	1.6E-01	3.9E-01	-0.25	dfrG
ARO_3000379	1.6E-01	3.9E-01	-0.14	OprM
ARO_3002687	1.7E-01	4.0E-01	-0.05	catQ
ARO_3002858	1.7E-01	4.0E-01	-0.03	dfrA12
ARO_3000347	1.7E-01	4.0E-01	-0.28	ErmA
ARO_3000807	1.6E-01	4.0E-01	-0.26	mexH
ARO_3000621	1.6E-01	4.0E-01	0.01	PC1 beta-lactamase (blaZ)
ARO_3003046	1.7E-01	4.0E-01	-0.03	qacA
ARO_3002965	1.6E-01	4.0E-01	-0.26	vanWG
ARO_3002943	1.7E-01	4.0E-01	0.30	vanHB
ARO_3000025	1.7E-01	4.0E-01	0.12	patB
ARO_3004073	1.7E-01	4.0E-01	-0.20	MuxA
ARO_3000368	1.8E-01	4.0E-01	-0.13	vanC
ARO_3002970	1.8E-01	4.1E-01	-0.11	vanTC
ARO_3003698	1.8E-01	4.1E-01	-0.21	mexP
ARO_3003835	1.9E-01	4.2E-01	-0.24	cdeA
ARO_3004077	1.9E-01	4.2E-01	-0.20	PmpM
ARO_3003551	1.9E-01	4.3E-01	-0.19	emeA
ARO_3002528	2.0E-01	4.3E-01	0.02	AAC(3)-Ia
ARO_3002578	2.0E-01	4.3E-01	-0.24	AAC(6')-Ib7
ARO_3002686	2.0E-01	4.3E-01	0.15	catP
ARO_3003693	2.0E-01	4.3E-01	-0.19	mexK
ARO_3002452	2.0E-01	4.3E-01	-0.79	OKP-B-19
ARO_3002957	2.0E-01	4.3E-01	-0.14	vanYD
ARO_3002921	2.0E-01	4.4E-01	-0.38	vanRB
ARO_3001338	2.1E-01	4.4E-01	-0.57	SHV-100
ARO_3004056	2.2E-01	4.5E-01	-0.10	ArmR
ARO_3002317	2.2E-01	4.5E-01	0.00	KPC-7
ARO_3002391	2.1E-01	4.5E-01	-0.94	OXY-1-3
ARO_3000567	2.1E-01	4.5E-01	0.97	tet(40)
ARO_3002185	2.2E-01	4.5E-01	-0.06	MOX-6
ARO_3002972	2.2E-01	4.5E-01	-0.11	vanTG
ARO_3001796	2.3E-01	4.6E-01	-0.23	OXA-50
ARO_3002956	2.3E-01	4.6E-01	-0.40	vanYB
ARO_3002999	2.3E-01	4.7E-01	0.10	CblA-1
ARO_3004036	2.3E-01	4.7E-01	0.02	tetB(60)
ARO_3000316	2.5E-01	5.0E-01	0.05	mphA
ARO_3003923	2.5E-01	5.0E-01	-0.89	oqxB
ARO_3002930	2.5E-01	5.0E-01	0.19	vanRO

ARO_3003022	2.9E-01	5.1E-01	0.01	dfrB3
ARO_3002705	2.9E-01	5.1E-01	-0.01	floR
ARO_3002213	2.9E-01	5.1E-01	-0.01	IMP-22
ARO_3004041	2.9E-01	5.1E-01	-0.48	Klebsiella pneumoniae acrA
ARO_3002482	2.9E-01	5.1E-01	-0.05	LRA-1
ARO_3002486	2.9E-01	5.1E-01	0.01	LRA-7
ARO_3001214	2.6E-01	5.1E-01	-0.56	mdtM
ARO_3000215	2.9E-01	5.1E-01	0.00	mecR1
ARO_3001610	2.9E-01	5.1E-01	0.01	OXA-243
ARO_3001503	2.9E-01	5.1E-01	0.03	OXA-258
ARO_3001780	2.7E-01	5.1E-01	0.08	OXA-85
ARO_3003836	2.9E-01	5.1E-01	0.01	qacH
ARO_3002828	2.7E-01	5.1E-01	-0.19	srmB
ARO_3000573	2.9E-01	5.1E-01	0.02	tet(43)
ARO_3000013	2.6E-01	5.1E-01	-0.26	vanB
ARO_3003727	2.9E-01	5.1E-01	0.00	vanKI
ARO_3003724	2.9E-01	5.1E-01	-0.01	vanWI
ARO_3002841	2.9E-01	5.1E-01	0.01	vatB
ARO_3004032	3.0E-01	5.2E-01	0.40	tetA(46)
ARO_3003033	3.1E-01	5.4E-01	-0.20	mexY
ARO_3004033	3.1E-01	5.4E-01	0.36	tetB(46)
ARO_3003209	3.2E-01	5.5E-01	-0.60	FosA5
ARO_3002538	3.8E-01	5.5E-01	0.01	AAC(3)-IIIc
ARO_3002540	3.8E-01	5.5E-01	0.00	AAC(3)-VIa
ARO_3002589	3.8E-01	5.5E-01	0.04	AAC(6')-IId
ARO_3001816	3.4E-01	5.5E-01	-0.24	ACC-2
ARO_3003848	3.8E-01	5.5E-01	-0.02	ADC-2
ARO_3000780	3.8E-01	5.5E-01	0.02	adel
ARO_3002646	3.8E-01	5.5E-01	0.01	APH(3')-IIc
ARO_3002660	3.8E-01	5.5E-01	-0.44	APH(6)-Id
ARO_3000838	3.8E-01	5.5E-01	-0.03	arlR
ARO_3000839	3.8E-01	5.5E-01	-0.02	arlS
ARO_3002847	3.8E-01	5.5E-01	-0.04	arr-2
ARO_3002854	3.2E-01	5.5E-01	-0.18	dfrA1
ARO_3003013	3.8E-01	5.5E-01	-0.03	dfrA15
ARO_3002860	3.4E-01	5.5E-01	0.14	dfrA17
ARO_3003953	3.8E-01	5.5E-01	0.01	hmrM
ARO_3002259	3.8E-01	5.5E-01	-0.02	IND-3
ARO_3003111	3.8E-01	5.5E-01	-0.15	IsaB
ARO_3000800	3.2E-01	5.5E-01	-0.17	MexC
ARO_3000808	3.5E-01	5.5E-01	-0.14	mexI
ARO_3003692	3.3E-01	5.5E-01	-0.16	mexJ
ARO_3003035	3.8E-01	5.5E-01	-0.01	mfpA

ARO_3004074	3.3E-01	5.5E-01	-0.08	MuxB
ARO_3000802	3.8E-01	5.5E-01	-0.12	OprJ
ARO_3000805	3.8E-01	5.5E-01	-0.09	OprN
ARO_3001710	3.4E-01	5.5E-01	0.18	OXA-211
ARO_3001398	3.8E-01	5.5E-01	0.02	OXA-3
ARO_3000822	3.6E-01	5.5E-01	0.03	pmrA
ARO_3002794	3.2E-01	5.5E-01	0.01	QnrS5
ARO_3001301	3.5E-01	5.5E-01	0.05	RlmA(II)
ARO_3003056	3.8E-01	5.5E-01	-0.01	smeE
ARO_3002493	3.8E-01	5.5E-01	-0.01	SRT-1
ARO_3000168	3.7E-01	5.5E-01	-0.04	tet(D)
ARO_3000178	3.8E-01	5.5E-01	0.01	tet(K)
ARO_3000186	3.4E-01	5.5E-01	-0.22	tetM
ARO_3000193	3.8E-01	5.5E-01	0.00	tetT
ARO_3000005	3.4E-01	5.5E-01	-0.20	vanD
ARO_3002934	3.6E-01	5.5E-01	-0.47	vanSD
ARO_3002935	3.8E-01	5.5E-01	-0.03	vanSE
ARO_3002953	3.8E-01	5.5E-01	-0.01	vanXM
ARO_3004075	3.8E-01	5.5E-01	-0.15	MuxC
ARO_3000410	3.9E-01	5.6E-01	0.20	sul1
ARO_3002598	4.0E-01	5.7E-01	-0.17	ANT(3'')-li-AAC(6')-Ild fusion protein
ARO_3001396	4.1E-01	5.8E-01	-0.32	OXA-1
ARO_3000616	4.2E-01	5.9E-01	0.12	mel
ARO_3004038	4.3E-01	6.0E-01	-0.20	Pseudomonas aeruginosa emrE
ARO_3000498	4.4E-01	6.1E-01	-1.33	ErmF
ARO_3004054	4.4E-01	6.2E-01	-0.11	Pseudomonas aeruginosa CpxR
ARO_3002982	4.6E-01	6.4E-01	-0.11	amrA
ARO_3000593	4.6E-01	6.4E-01	0.19	ErmQ
ARO_3000801	4.6E-01	6.4E-01	-0.18	MexD
ARO_3003839	4.7E-01	6.4E-01	0.11	Mrx
ARO_3003680	4.8E-01	6.6E-01	-0.25	TriB
ARO_3003030	5.2E-01	7.0E-01	-0.17	mexV
ARO_3000167	5.3E-01	7.1E-01	0.02	tet(C)
ARO_3001307	5.3E-01	7.1E-01	-0.02	VgbA
ARO_3003954	5.4E-01	7.2E-01	0.00	efmA
ARO_3000804	5.4E-01	7.2E-01	0.04	MexF
ARO_3000412	5.5E-01	7.3E-01	-0.25	sul2
ARO_3003583	5.5E-01	7.3E-01	-0.19	basS
ARO_3002684	5.6E-01	7.3E-01	-0.03	catII
ARO_3003948	5.5E-01	7.3E-01	-0.04	efrA
ARO_3000230	5.6E-01	7.3E-01	0.00	ANT(2'')-Ia

ARO_3002966	5.8E-01	7.5E-01	-0.15	vanXYC
ARO_3000190	5.8E-01	7.5E-01	0.35	tetO
ARO_3002645	5.9E-01	7.5E-01	-0.11	APH(3')-IIb
ARO_3003097	5.9E-01	7.5E-01	-0.12	CfxA6
ARO_3000522	5.9E-01	7.5E-01	-0.53	ErmG
ARO_3000595	5.9E-01	7.5E-01	-0.30	ErmT
ARO_3002881	6.0E-01	7.5E-01	0.03	lmrC
ARO_3002895	6.0E-01	7.5E-01	-0.16	SAT-1
ARO_3003070	6.0E-01	7.5E-01	-0.13	vanXD
ARO_3002535	6.1E-01	7.5E-01	-0.22	AAC(3)-IIc
ARO_3002539	6.1E-01	7.5E-01	-0.29	AAC(3)-IV
ARO_3000174	6.1E-01	7.5E-01	0.01	tet(G)
ARO_3002944	6.1E-01	7.6E-01	-0.03	vanHD
ARO_3003717	6.4E-01	7.8E-01	-0.05	ESP-1
ARO_3000181	6.4E-01	7.8E-01	0.01	tet(V)
ARO_3003710	6.5E-01	7.9E-01	-0.06	mexL
ARO_3000165	6.5E-01	7.9E-01	-0.15	tet(A)
ARO_3002931	6.5E-01	7.9E-01	-0.03	vanSA
ARO_3000010	6.7E-01	8.1E-01	-0.03	vanA
ARO_3002605	6.7E-01	8.1E-01	0.00	aadA5
ARO_3001933	7.1E-01	8.5E-01	-0.63	CTX-M-72
ARO_3000175	7.1E-01	8.5E-01	0.01	tet(H)
ARO_3002556	7.1E-01	8.5E-01	0.05	AAC(6')-II
ARO_3002680	7.2E-01	8.6E-01	-0.25	catB8
ARO_3000809	7.3E-01	8.6E-01	-0.14	opmD
ARO_3002897	7.3E-01	8.6E-01	-0.37	SAT-4
ARO_3001313	7.3E-01	8.6E-01	0.00	facT
ARO_3003197	7.4E-01	8.7E-01	-0.06	aadA25
ARO_3003949	7.7E-01	9.0E-01	0.00	efrB
ARO_3003682	7.7E-01	9.0E-01	-0.13	OpmH
ARO_3001959	7.8E-01	9.0E-01	-0.45	CTX-M-100
ARO_3002942	7.9E-01	9.1E-01	-0.02	vanHA
ARO_3003559	8.1E-01	9.3E-01	-0.29	cepA beta-lactamase
ARO_3002628	8.2E-01	9.4E-01	-0.02	aad(6)
ARO_3000596	8.2E-01	9.4E-01	0.02	ErmX
ARO_3002949	8.2E-01	9.4E-01	-0.05	vanXA
ARO_3002837	8.3E-01	9.4E-01	-0.30	lnuC
ARO_3002756	8.3E-01	9.4E-01	0.21	QnrB41
ARO_3002875	8.4E-01	9.4E-01	0.04	dfrE
ARO_3002647	8.5E-01	9.5E-01	0.00	APH(3')-IIIa
ARO_3000179	8.5E-01	9.5E-01	-0.21	tet(L)
ARO_3002069	8.6E-01	9.6E-01	-0.09	CMY-59
ARO_3002019	9.0E-01	9.8E-01	0.01	CMY-8
ARO_3003095	9.0E-01	9.8E-01	0.12	imiS

ARO_3002225	9.0E-01	9.8E-01	-0.03	IMP-34
ARO_3001413	9.0E-01	9.8E-01	0.01	OXA-18
ARO_3000565	9.0E-01	9.8E-01	0.00	tet(38)
ARO_3000177	8.8E-01	9.8E-01	-0.25	tet(J)
ARO_3002882	9.1E-01	9.8E-01	0.07	lmrD
ARO_3002363	9.1E-01	9.8E-01	0.02	PER-1
ARO_3003069	9.1E-01	9.8E-01	-0.02	vanXYG
ARO_3002819	9.2E-01	9.9E-01	-0.03	msrC
ARO_3003210	9.4E-01	9.9E-01	-0.27	FosA4
ARO_3000026	9.4E-01	9.9E-01	-0.01	mepA
ARO_3000746	9.4E-01	9.9E-01	0.00	mepR
ARO_3000815	9.4E-01	9.9E-01	0.00	mgrA
ARO_3003199	1.0E+00	1.0E+00	0.02	AAC(6')-Iak
ARO_3002613	9.6E-01	1.0E+00	0.01	aadA13
ARO_3002641	9.7E-01	1.0E+00	-0.07	APH(3')-Ia
ARO_3002655	1.0E+00	1.0E+00	-0.24	APH(4)-Ia
ARO_3003730	1.0E+00	1.0E+00	-0.06	Bifidobacteria intrinsic ileS conferring resistance to mupirocin
ARO_3001920	9.8E-01	1.0E+00	-0.09	CTX-M-59
ARO_3003955	9.9E-01	1.0E+00	-0.03	efpA
ARO_3000375	9.6E-01	1.0E+00	0.07	ErmB
ARO_3004085	1.0E+00	1.0E+00	-0.06	InuG
ARO_3002665	9.9E-01	1.0E+00	-0.16	npmA
ARO_3003700	9.9E-01	1.0E+00	-0.18	opmE
ARO_3002788	1.0E+00	1.0E+00	-0.04	QnrD1
ARO_3001388	9.6E-01	1.0E+00	-0.37	TEM-211
ARO_3000556	9.5E-01	1.0E+00	-0.01	tet44
ARO_3004035	9.7E-01	1.0E+00	-0.01	tetA(60)
ARO_3002909	1.0E+00	1.0E+00	0.00	vanG
ARO_3002919	9.9E-01	1.0E+00	-0.08	vanRA
ARO_3002922	1.0E+00	1.0E+00	-0.06	vanRC
ARO_3002955	9.5E-01	1.0E+00	0.01	vanYA
ARO_3002962	1.0E+00	1.0E+00	-0.09	vanZA

Table 2 – Engraftment of spores is associated with significant reduction in antibiotic resistance genes

Drug Class	log2FC high engrafters - log2 Placebo	p-value	FDR	Examples of antibiotics in drug class
Aminocoumarin antibiotic	-1.534	0.003	0.035	Novobiocin, Albamycin, Coumemycin, Clorobiocin
Aminoglycoside antibiotic	-1.241	0.009	0.035	Kanamycin, Amikacin, Tobramycin, Dibekacin, Gentamicin, Sisomicin, Netilmicin, Neomycins (B, C, E) Streptomycin
cephalosporin	-1.428	0.014	0.035	Cefacettrill, Cefradrin, Cefroxadin, Cefaloglycin, Cefaclor, Cefalexin, Cefadroxil, Cefatrizin, Cefazedon, Cefapirin, Ceftezol, Cefazolin, Cefazaflur, Cefalotin, Cefaloridin, Cefalonium
cephamycin	-1.214	0.008	0.035	Cefoxitin, Cefotetan, Cefmetazole
Fluoroquinolone antibiotic	-1.401	0.007	0.035	Flumequine, oxolinic acid, rosoxacin, ciprofloxacin, fleroxacin, lomefloxacin, nadifloxacin, norfloxacin, ofloxacin, pefloxacin, rufloxacin, balofloxacin, grepafloxacin, levofloxacin, pazufloxacin, sparfloxacin, temafloxacin, clinafloxacin, gatifloxacin, moxifloxacin, sitafloxacin, prulifloxacin, besifloxacin, delafloxacin, ozenoxacin, danofloxacin, difloxacin, enrofloxacin, ibafloxacin, marbofloxacin, orbifloxacin, sarafloxacin
glycylcycline	-1.658	0.009	0.035	Tigercycline
Macrolide antibiotic	-1.288	0.002	0.035	Erythromycin, Clarithromycin, Roxithromycin
Nitroimidazole antibiotic	-1.477	0.013	0.035	Nitroimidazole

Nucleoside antibiotic	-1.347	0.011	0.035	Tunicamycin, Streptothricin
penam	-1.424	0.010	0.035	Benzylpenicillin
Peptide antibiotic	-1.279	0.006	0.035	
Phenicol antibiotic	-1.139	0.012	0.035	chloramphenicol
Rifamycin antibiotic	-1.470	0.006	0.035	rifamycin
Benzalkonium chloride	-1.016	0.019	0.035	Benzalkonium chloride
monobactam	-1.114	0.018	0.035	Aztreonam
penem	-1.155	0.015	0.035	Faropenem
rhodamine	-1.016	0.019	0.035	rhodamine
triclosan	-1.234	0.018	0.035	triclosan antibiotics
Lincosamide antibiotic	-1.123	0.021	0.037	lincomycin, clindamycin, pirlimycin
Streptogramin antibiotic	-1.065	0.035	0.057	streptogramin
Acridine dye	-0.905	0.043	0.067	
carbapenem	-1.239	0.052	0.075	carbapenem
Tetracycline antibiotic	-0.558	0.050	0.075	tetracycline
fosfomycin	-1.024	0.066	0.091	fosfomycin

Example 2: Bacteria associated with changes in abundance of Abx resistance genes

Computational analysis to determine whether the presence of specific bacterial species is predictive of a decrease in the abundance of antibiotic resistance genes was performed. Species identified in these analyses can be used in designed Abx compositions. To identify microbial species that are associated with a reduction of antimicrobial resistance genes, we calculated the Spearman correlation between species abundance and the DCMA of drug classes showing a significant reduction in subjects in whom engraftment of spores was observed relative to placebo. P-values were adjusted to account for multiple hypothesis testing using the Benjamini-Hochberg procedure ($p < 0.1$) as described *supra*. The set of species associated with significant reduction of at least one drug of antibiotic resistance genes is summarized in Table 3. We have further defined the set of species positively correlated with elevated abundances of at least one class of antibiotic resistance genes, summarized in Table 4. Dose species in all tables are bacterial species identified in dose lots administered to subjects. Full length 16S sequences of species described herein are provided in Tables 5 (negative correlates) and 6 (positive correlates). In some embodiments, the bacterium(a) are from a genus listed below. Those of skill in the art can use standard methods to identify the genera.

Table 3 – Species significantly correlated with reduction of at least one class of antibiotic resistance genes

Species	Number of drug classes with significant reduction	Spore-former (Y/N)	Dose Species (Y/N)	Exemplary sequence identifier	Representative Genome Accession Number(s) (NCBI)
Anaerofustis_stercorihominis	21	Y	Y	96, 97	GCF_000154825
Anaerostipes_caccae	20	Y	N	62-66	GCF_000154305
Anaerostipes_hadrus	23	Y	Y	223	GCF_000332875
Anaerostipes_sp.	24	Y	Y	24	GCA_900066985
Anaerostipes_unclassified	21	N	N		
Anaerotruncus_colihominis	24	Y	Y	89-92	GCF_000154565
Anaerotruncus_unclassified	22	N	N		
Bacteroides_fragilis	18	N	N	184-191	GCF_000273095
Bacteroides_uniformis	6	N	N	192-198	GCF_000273275
Bacteroides_vulgatus	16	N	N	199-204	GCF_000273295
Blautia_faecis (a)	22	Y	Y	9	GCA_900066145
Blautia_faecis (b)	24	Y	Y	22	GCA_900066945
Blautia_hydrogenotrophica	22	Y	Y	101	GCF_000157975
Blautia_producta	21	Y	Y	281	GCF_000373885
Blautia_sp.	22	Y	Y	268, 269	
Blautia_sp_CAG_257	22	Y	Y	261	
Blautia_wexlerae	24	Y	Y	104-110, 255	GCF_000159975
Clostridiaceae_bacterium_JC118	21	Y	Y	222	GCF_000313565
Clostridiales_bacterium_1_7_47FAA	23	Y	Y	98	GCF_000155435
Clostridium_aldenense	21	Y	Y	278	
Clostridium_asparagiforme	22	Y	Y	103	GCF_000158075
Clostridium_bartlettii	24	Y	Y	80-85	GCF_000154445
Clostridiumbolteae	23	Y	Y	73	GCF_000154365
Clostridium_clostridioforme	21	Y	Y	231-233	GCF_000371525
Clostridium_hathewayi	24	Y	Y	229, 230	GCF_000371445
Clostridium_lavalense	20	Y	Y	280	
Clostridium_leptum	22	Y	Y	71, 72	GCF_000154345
Clostridium_nexile	24	Y	N	282	GCF_000156035
Clostridium_ramosum	21	Y	Y	86	GCF_000154485
Clostridium_sp.	21	Y	Y	21	GCA_900066915
Clostridium_sp.	22	Y	Y	18	GCA_900066805
Clostridium_sp.	22	Y	Y	6	GCA_900066055
Clostridium_sp.	22	Y	Y	12	GCA_900066535
Clostridium_sp.	23	Y	Y	11	GCA_900066365
Clostridium_sp.	24	Y	Y	8	GCA_900066135
Clostridium_sp.	24	Y	Y		
Clostridium_sp.	23	Y	Y	17	GCA_900066795
Clostridium_sp.	22	Y	Y	15, 16	GCA_900066785

Clostridium_sp.	23	Y	Y	10	GCA_900066155
Clostridium_scindens	21	Y	Y	87, 88	GCF_000154505
Clostridium_sp_CAG_91	23	Y	Y	266	
Clostridium_sp_KLE_1755	22	Y	N	247	GCF_000466465
Clostridium_symbiosum	23	Y	Y	248	GCF_000466485
Coprobacillus_unclassified	21	N	N		
Coprococcus_catus	22	Y	Y	143	GCF_000210555
Coprococcus_comes	21	Y	Y	99	GCF_000155875
Dialister_invisus	21	N	N	111-113	GCF_000160055
Dorea_formicigenerans	22	Y	Y	114-119	GCF_000169235
Dorea_longicatena	21	Y	Y	60, 61	GCF_000154065
Eggerthella_lenta	22	N	N	39-41	GCF_000024265
Eggerthella_unclassified	20	N	N		
Emergencia_timonensis	22	Y	Y	14	GCA_900066695
Erysipelotrichaceae_bacterium_21_3	21	N	N	164-168	GCF_000242195
Erysipelotrichaceae_bacterium_6_1_45	21	N	N	160-163	GCF_000242175
Eubacterium_contortum	23	Y	Y	272-275	
Eubacterium_eligens	22	Y	Y	43-47	GCF_000146185
Eubacterium_hallii	22	Y	Y	120	GCF_000173975
Eubacterium_ramulus	22	Y	Y	249	GCF_000469345
Eubacterium_rectale	23	Y	Y	34-38	GCF_000020605
Eubacterium_sp.	21	Y	Y		
Eubacterium_sp_3_1_31	20	Y	Y	205-209, 262	GCF_000273585
Eubacterium_ventriosum	23	Y	Y	48	GCF_000153885
Faecalibacterium_prausnitzii	24	Y	Y	74-79	GCF_000154385
Flavonifractor_plautii	23	Y	Y	158	GCF_000239295
Flavonifractor_sp. (a)	21	Y	Y	19	GCA_900066825
Flavonifractor_sp. (b)	23	Y	Y	13	GCA_900066645
Fusicatenibacter_saccharivorans	23	Y	Y	4	GCA_001405555
Gemmiger_formicilis_(a)	24	Y	Y	256	
Gemmiger_formicilis_(b)	24	Y	Y	263	
Holdemania_filiformis	24	Y	Y	102	GCF_000157995
Holdemania_unclassified	24	N	N		
Intestinimonas_butyrificiproducens	23	Y	Y	264	
Intestinimonas_massiliensis	22	N	N	20	GCA_900066895
Lachnospira_pectinoschiza	24	Y	Y	5	GCA_001405835
Lachnospira_pectinoschiza	19	Y	Y	270	
Lachnospiraceae_bacterium_1_1_57FAA	23	Y	Y	150	GCF_000218445
Lachnospiraceae_bacterium_1_4_56FAA	21	Y	Y	145	GCF_000218385
Lachnospiraceae_bacterium_2_1_58FAA	23	Y	Y	151	GCF_000218465
Lachnospiraceae_bacterium_3_1_46FAA	22	Y	Y	139	GCF_000209405
Lachnospiraceae_bacterium_3_1_57FAA_CT1	22	Y	Y	146-148	GCF_000218405
Lachnospiraceae_bacterium_4_1_37FAA	18	Y	N	137	GCF_000191805

Lachnospiraceae_bacterium_5_1_57FAA	22	Y	Y	149	GCF_000218425
Lachnospiraceae_bacterium_5_1_63FAA	24	Y	Y	134	GCF_000185525
Lachnospiraceae_bacterium_7_1_58FAA	23	Y	Y	159	GCF_000242155
Lachnospiraceae_bacterium_8_1_57FAA	21	Y	Y	135	GCF_000185545
Lachnospiraceae_bacterium_9_1_43BFAA	24	Y	Y	141	GCF_000209445
Lactonifactor_longoviformis	23	Y	Y	271	
Oscillibacter_unclassified	23	N	N		
Oscillospiraceae_bacterium_VE202_24	23	N	N	265	
Parabacteroides_unclassified	18	N	N		
Prevotella_buccae	22	N	N	133	GCF_000184945
Roseburia_faecis	23	Y	Y	259	
Roseburia_hominis	21	Y	Y	152-155	GCF_000225345
Roseburia_intestinalis	23	Y	Y	100	GCF_000156535
Roseburia_inulinivorans	24	Y	Y	283	GCF_000174195
Ruminococcus_bicirculans	23	Y	Y	25, 26	GCA_900067025
Ruminococcus_bromii	22	Y	Y	142	GCF_000209875
Ruminococcus_gnavus	23	Y	Y	250-254, 277	GCF_000507805
Ruminococcus_obeum_(a)	23	Y	Y	49-53	GCF_000153905
Ruminococcus_obeum_(b)	19	Y	Y	267	
Ruminococcus_sp.	22	Y	Y	7, 258	GCA_900066095
Ruminococcus_torques	24	Y	Y	54-59	GCF_000153925
Streptococcus_parasanguinis	21	N	N	170-173	GCF_000262145
Streptococcus_salivarius	21	N	N	210-216	GCF_000286295
Subdoligranulum_unclassified	23	N	N		
Sutterella_wadsworthensis	9	N	N	243-246	GCF_000411515
Bacteroides_thetaiotaomicron	1	N	N	239-242	GCF_000403155
Haemophilus_parainfluenzae	7	N	N	169	GCF_000261285
Parabacteroides_distasonis	2	N	N	217-221	GCF_000307435
Akkermansia_muciniphila	17	N	N	31-33	GCF_000020225
Bacteroides_dorei	11	N	N	174-183	GCF_000273055
Butyrivibrio_sp.	15	N	N	260	
Clostridium_citroniae	20	Y	Y	157	GCF_000233455
Clostridium_difficile	8	Y	N	1-3	GCA_000451045
Clostridium_disporicum	9	Y	Y	23	GCA_900066955
Clostridium_innocuum	4	Y	Y	224-228	GCF_000371425
Clostridium_spiroforme	11	Y	Y	93-95	GCF_000154805
Dorea_unclassified	13	N	N		
Eisenbergiella_massiliensis	11	Y	Y	257	
Eisenbergiella_tayi_sp.	14	Y	Y	276	
Erysipelotrichaceae_bacterium_2_2_44A	20	N	N	156	GCF_000225685
Eubacterium_siraeum	14	Y	Y	67-70	GCF_000154325
Lachnospiraceae_bacterium_6_1_63FAA	11	Y	N	140	GCF_000209425
Ruminococcaceae_bacterium_D16	19	Y	Y	121	

Ruminococcus_gnavus	7	Y	Y	250-254, 277	GCF_000507805
Ruminococcus_sp.	12	Y	Y	7, 258	GCF_000507805
Streptococcus_australis	4	N	N	136	GCF_000186465
Streptococcus_gordonii	3	N	N	27-30	GCF_000017005
Streptococcus_infantis	5	N	N	284-286	GCF_000215385, GCF_000187465, GCF_000223335, GCF_000223255, GCF_000260755
Streptococcus_sanguinis	5	N	N	138	GCF_000192205
Dysgonomonas_mossii	6	N	N	144	GCF_000213575
Streptococcus_thermophilus	1	N	N	128-132	GCF_000182875
Peptostreptococcaceae_noname_unclassified	10	N	N		
Turicibacter_unclassified	6	N	N		
Burkholderiales_bacterium_1_1_47	3	N	N	42	GCF_000144975
Clostridium_sp_CL_6	4	Y	N	287	
Desulfovibrio_desulfuricans	1	N	N		GCF_000384815, GCF_000022125, GCF_000420465, GCF_000189295
Lactobacillus_fermentum	1	N	N	234-238	GCF_000397165
Streptococcus_vestibularis	1	N	N	122-127	GCF_000180075

Table 4 – Species significantly positively correlated with elevated abundances of at least one class of antibiotic resistance genes

Species	Number of drug classes associated with increasing species abundance	Spore-former (Y/N)	Dose Species (Y/N)	Exemplary sequence identifier	Representative Genome Accession Number(s) (NCBI)
Campylobacter_curvus	20	N	N	367	GCF_000376325
Citrobacter_freundii	22	N	N	361, 362	GCF_000312465
Citrobacter_unclassified	22	N	N		
Desulfovibrio_desulfuricans	6	N	N		GCF_000384815, GCF_000022125, GCF_000420465, GCF_000189295
Enterobacter_cloacae	22	N	N	387	GCF_000467655
Enterococcus_faecalis	21	N	N		GCF_000393515, GCF_000394095, GCF_000393615, GCF_000157495, GCF_000390625, GCF_000390925, GCF_000148005, GCF_000148065, GCF_000394075, GCF_000393495, GCF_000391465, GCF_000392675, GCF_000396365, GCF_000157475, GCF_000396665, GCF_000148245, GCF_000393155, GCF_000148165, GCF_000391165, GCF_000157515, GCF_000396085, GCF_000395285, GCF_000479065, GCF_000393095, GCF_000393255, GCF_000391065, GCF_000394955, GCF_000415065, GCF_000394975, GCF_000415085, GCF_000395405,

					GCF_000172575, GCF_000390525, GCF_000393115, GCF_000394775, GCF_000393475, GCF_000390705, GCF_000205205, GCF_000395135, GCF_000392815, GCF_000320305, GCF_000396205, GCF_000390665, GCF_000390685, GCF_000391725, GCF_000163815, GCF_000391605, GCF_000391445, GCF_000391425, GCF_000396185, GCF_000148445, GCF_000148125, GCF_000396605, GCF_000396045, GCF_000291565, GCF_000394315, GCF_000395035, GCF_000147455, GCF_000391345, GCF_000396525, GCF_000392755
Escherichia_coli	24	N	N	388-394	GCF_000474825
Escherichia_unclassified	23	N	N		
Fusobacterium_nucleatum	15	N	N	403	GCF_000479185
Klebsiella_oxytoca	22	N	N	330	GCF_000252915
Klebsiella_pneumoniae	22	N	N	395-402	GCF_000474865
Klebsiella_unclassified	21	N	N		
Lactobacillus_animalis	22	N	N	317	GCF_000183825
Lactobacillus_casei_paracasei	22	N	N	378, 379, 381-385	GCF_000409835, GCF_000418515
Lactobacillus_plantarum	22	N	N	386	GCF_000463075
Lactobacillus_rhamnosus	24	N	N	328, 329	GCF_000235785
Lactobacillus_salivarius	9	N	N	303	GCF_000159395
Lactococcus_lactis	15	N	N	363, 364	GCF_000348965

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Megasphaera_micronuciformis	22	N	N	310	GCF_000165735
Megasphaera_unclassified	11	N	N		
Morganella_morganii	24	N	N	355	GCF_000286435
Pantoea_unclassified	24	N	N		
Pediococcus_acidilactici	19	N	N	299	GCF_000146325
Proteus_penneri	17	N	N	301, 302	GCF_000155835
Streptococcus_mitis_oralis_pneumoniae	3	N	N	288-291, 332	GCF_000014365, GCF_000257865
Streptococcus_thermophilus	5	N	N	312-316	GCF_000182875
Veillonella_atypica	19	N	N	311	GCF_000179755
Veillonella_dispar	18	N	N	304-306	GCF_000160015
Veillonella_parvula	13	N	N	322	GCF_000215025
Bifidobacterium_dentium	1	N	N	300	GCF_000146775
Butyrivibrio_sp.	1	N	N	404	
Clostridium_sp_CL_6	1	Y	N	287	NCBI Taxonomy ID 1499683
Enterococcus_faecium	18	N	N	380	GCF_000414965
Erysipelotrichaceae_bacterium_2_2_44A	1	N	N	326	GCF_000225685
Lactobacillus_fermentum	2	N	N	368-372	GCF_000397165
Lactobacillus_gasseri	1	N	N	377	GCF_000406345
Proteus_mirabilis	8	N	N	292-298	GCF_000069965
Rothia_dentocariosa	5	N	N	307-309	GCF_000164695
Proteus_unclassified	5	N	N		
Veillonella_unclassified	14	N	N		
Alistipes_nderdonkii	1	N	N	365, 366	GCF_000374505
Bacteroides_ovatus	5	N	N	333-336	GCF_000273195
Bacteroides_vulgatus	4	N	N	344-349	GCF_000273295
Bilophila_unclassified	3	N	N		
Bilophila_wadsworthia	3	N	N	318-321	GCF_000185705
Parabacteroides_distasonis	6	N	N	356-360	GCF_000307435
Parabacteroides_unclassified	3	N	N		

Alistipes_unclassified	2	N	N		
Rothia_mucilaginosa	1	N	N	327	GCF_000231235
Bacteroides_thetaiotaomicron	1	N	N	373-376	GCF_000403155
Bacteroides_uniformis	2	N	N	337-343	GCF_000273275
Eggerthella_unclassified	1	N	N		
Eubacterium_sp_3_1_31	2	Y	Y	350-354	GCF_000273585
Lachnospiraceae_bacterium_3_1_57FAA_CT1	1	Y	Y	323-325	GCF_000218405
Ruminococcus_obeum	1	Y	Y	405	
Turicibacter_unclassified	1	N	N		
Clostridium_perfringens	1	Y	Y	331	GCF_000255475

Table 5 - Full length 16S rDNA sequences of species (negative correlates)

Species Name	Sequence Identifier	16S rDNA sequence
Clostridium_difficile	SEQ ID NO: 1	ttatgagagtttgatcctggctcaggacgaacgctggcggcgtgcctaatacatgcaagtcgaacgctcttttccaccggagc ttgctccaccggaaaaagaggagtggcgaacgggtgagtaaacacgtgggtaacctgccatcagaaggggataaaccttgg aaacagggtgctaataccgtataacaatcgaaccgcattggtttgattgaaaggcgcttcgggtgctgctgtagtgatggacc gcgggtgcattagctagtgtgaggtaacggctcaccaggccacgatgcataccgacctgagagggtgacggccacatt gggactgagacacggccaaactcctacgggagggcagcagtagggaatcttcggcaatggacgaaagtctgaccgagcaa cgccgcgtgagtgaaagaagggtttcggatcgtaaaactctgtgttagagaagaacaaggatgagagtaactgttcacccctga cggtatctaaccagaagccacggctaactacgtgccagcagccgggtaatacgtaggtggcaagcggtgtccggatttatt gggcgtaaaagcagcgcaggcggtttcttaagtctgatgtgaaagccccggctcaaccggggagggtcattggaactgg gagacttgagtcagaagaggagagtggaattccatgtgtagcgggtgaaatgcgtagatatatggaggaaacaccagtggcga aggcggtctctgtgtgtaactgacgctgaggctcgaagcggtggggagcaaacaggattagataccctggtagtcacgc cgtaaacgatgagtgctaagtgttgagggtttccgcccttca
Clostridium_difficile	SEQ ID NO: 2	ttatgagagtttgatcctggctcaggacgaacgctggcggcgtgcctaatacatgcaagtcgaacgctcttttccaccggagc ttgctccaccggaaaaagaggagtggcgaacgggtgagtaaacacgtgggtaacctgccatcagaaggggataaaccttgg aaacagggtgctaataccgtataacaatcgaaccgcattggtttgattgaaaggcgcttcgggtgctgctgtagtgatggacc gcgggtgcattagctagtgtgaggtaacggctcaccaggccacgatgcataccgacctgagagggtgacggccacatt gggactgagacacggccaaactcctacgggagggcagcagtagggaatcttcggcaatggacgaaagtctgaccgagcaa cgccgcgtgagtgaaagaagggtttcggatcgtaaaactctgtgttagagaagaacaaggatgagagtaactgttcacccctga cggtatctaaccagaagccacggctaactacgtgccagcagccgggtaatacgtaggtggcaagcggtgtccggatttatt gggcgtaaaagcagcgcaggcggtttcttaagtctgatgtgaaagccccggctcaaccggggagggtcattggaactgg gagacttgagtcagaagaggagagtggaattccatgtgtagcgggtgaaatgcgtagatatatggaggaaacaccagtggcga aggcggtctctgtgtgtaactgacgctgaggctcgaagcggtggggagcaaacaggattagataccctggtagtcacgc cgtaaacgatgagtgctaagtgttgagggtttccgcccttca
Clostridium_difficile	SEQ ID NO: 3	ttatgagagtttgatcctggctcaggacgaacgctggcggcgtgcctaatacatgcaagtcgaacgctcttttccaccggagc ttgctccaccggaaaaagaggagtggcgaacgggtgagtaaacacgtgggtaacctgccatcagaaggggataaaccttgg aaacagggtgctaataccgtataacaatcgaaccgcattggtttgattgaaaggcgcttcgggtgctgctgtagtgatggacc gcgggtgcattagctagtgtgaggtaacggctcaccaggccacgatgcataccgacctgagagggtgacggccacatt gggactgagacacggccaaactcctacgggagggcagcagtagggaatcttcggcaatggacgaaagtctgaccgagcaa cgccgcgtgagtgaaagaagggtttcggatcgtaaaactctgtgttagagaagaacaaggatgagagtaactgttcacccctga cggtatctaaccagaagccacggctaactacgtgccagcagccgggtaatacgtaggtggcaagcggtgtccggatttatt gggcgtaaaagcagcgcaggcggtttcttaagtctgatgtgaaagccccggctcaaccggggagggtcattggaactgg gagacttgagtcagaagaggagagtggaattccatgtgtagcgggtgaaatgcgtagatatatggaggaaacaccagtggcga aggcggtctctgtgtgtaactgacgctgaggctcgaagcggtggggagcaaacaggattagataccctggtagtcacgc cgtaaacgatgagtgctaagtgttgagggtttccgcccttca

		cggfatctaaccagaaagccacggctaactacgtgccagcagccgcggtaatacgtaggtggcaagcgttgcggatttatt gggcgtaaagcagcgcagcgggtttcttaagtctgatgtgaaagcccccggctcaaccggggagggcattggaactgg gagacttgatgcagaagaggagagtggaattccatgtgtagcgggtgaaatgcgtagatatattgaggaacaccagtggcga aggcggctctgtgtgttaactgacgtgaggctcgaagcgtggggagcaaacaggattagataccctggtagtccacgc cgtaacgatgagtgctaagtgttgagggtttccgccctca
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Streptococ- us_infantis	SEQ ID NO: 285	TGCCTAATACATGCAAGTAGAACGCTGAAGGAGGAGCTTGCTCTTCTGGATG AGTTGCGAACGGGTGAGTAACGCGTAGGTAACCTGCCTGGTAGCGGGGGAT AACTATTGAAAACGATAGCTAATACCGCATAACAGTAGATATCGCATGATA GCTGCTTGAAAGGTGCAATTGCACCACTACCAGATGGACCTGCGTTGTATTA GCTAGTTGGTGAGGTAACGGCTCACCAAGGCAACGATACATAGCCGACCTG AGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAGACTCCTACGGG AGGCAGCAGTAGGGAATCTTCGGCAATGGACGGAAGTCTGACCGAGCAACG CCGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAAGCTCTGTTGTAAGAGAAG AACGAGTGTGAGAGTGGAAAGTTTACACTGTGACGGTATCTTACCAGAAAG GGACGGCTAACTACGTGCCAGCAGCCGCGTAATACGTAGGTCCCGAGCGT TGTCGGATTATTGCGGCGTAAAGCGAGCGCAGGCGGTTAGATAAGTCTGA AGTTAAAGGCTGTGGCTTAACCATAGTACGCTTTGGAAACTGTTAACTTGA GTGCAAGAGGGGAGAGTGGAAATCCATGTGTAGCGGTGAAATGCGTAGATA TATGGAGGAACACCGGTGGCGAAAGCGGCTCTCTGGCTTGTAAGTACGNG AGGCTCGAAAGCGTGGGGAGCAAACAGGATTAGATACCTGGTAGTCCACG CCGTAAACGATGAGTGCTAGGTGTTAGACCCTTTCCGGGGTTTAGTGCCGTA GCTAACGCATTAAGCACTCCGCCTGGGGAGTACGACCGCAAGGTTGAAACT CAAAGGAATTGACGGGGGGCCCGCACAAAGCGGTGGAGCATGTGGTTTAATTC GAAGCAACGCGAAGAACCTTACCAGGTCTTGACATCCCTCTGACCGCTCTAG AGATAGAGTTTTCTTCGGGACAGAGGTGACAGGTGGTGCATGGTTGTCGTC AGCTCGTGTCGTGAGATGTTGGGTAAAGTCCCGCAACGAGCGCAACCCCTAT TGTTAGTTGCCATCATTCAGTTGGGCACTCTAGCGAGACTGCCGGTAATAAA CCGGAGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGACCTGGG CTACACACGTGCTACAATGGTTGGTACAACGAGTCGCAAGCCGGTGACGGC AAGCTAATCTCTTAAAGCCAATCTCAGTTCGGATTGTAGGCTGCAACTCGCC TACATGAAGTCGGAATCGCTAGTAATCGCGGATCAGCACGCCGCGGTGAAT ACG
Streptococ- us_infantis	SEQ ID NO: 286	GACGAACGCTGGCGGCGTGCCTAATACATGCAAGTAGAACGCTGAAGGAGG AGCTTGCTCTTCTGGATGAGTTGCGAACGGGTGAGTAACGCGTAGGTAACCT GCCTGGTAGCGGGGGATAACTATTGAAAACGATAGCTAATACCGCATAACA GTAGATATCGCATGATAGCTGCTTGAAAGGTGCAATTGCACCACTACCAGAT GGACCTGCGTTGTATTAGCTAGTTGGTGAGGTAACGGCTCACCAAGGCAAC GATACATAGCCGACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACG

		GCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATCTTCGGCAATGGACGGA AGTCTGACCGAGCAACGCCGCGTGAGTGAAGAAGGTTTTTCGGATCGTAAAG CTCTGTTGTAAGAGAAGAACGAGTGTGAGAGTGGAAGTTACACTGTGAC GGTATCTTACCAGAAAGGGACGGCTAACTACGTGCCAGCAGCCGCGGTAAT ACGTAGGTCCCAGCGTTGTCCGGATTTATTGGGCGTAAAGCGAGCGCAGG CGGTTAGATAAGTCTGAAGTTAAAGGCTGTGGCTTAACCATAGTACGCTTTG GAAACTGTTTAACTTGAGTGCAAGAGGGGAGAGTGGAATTCATGTGTAGC GGTGAAATGCGTAGATATATGGAGGAACACCGGTGGCGAAAGCGGCTCTCT GGCTTGTAAGTACGCTGAGGCTCGAAAGCGTGCGGAGCAAACAGGATTAG ATACCCTGGTAGTCCACGCCGTAAACGATGAGTGCTAGGTGTTAGACCCTTT CCGGGGTTTAGTGCCGAAGCTAACGCATTAAGCACTCCGCTGGGGAGTAC GACCGCAAGGTTGAACTCAAAGGAATTGACGGGGGCGCCGACAAGCGGTG GAGCATGTGGTTTAATTGCAAGCAACGCAAGAACCTTACCAGGTCTTGAC ATCCCTCTGACCGCTCTAGAGATAGAGTTTTCTTCGGGACAGAGGTGACAG GTGGTGATGGTTGTCTGTCAGCTCGTGTCTGAGATGTTGGGTTAAGTCCCG CAACGAGCGCAACCCCTATTGTTAGTTGCCATCATTTAGTTGGGCACTCTAG CGAGACTGCCGTAATAAACCGGAGGAAGGTGGGGATGACGTCAAATCATC ATGCCCTTATGACCTGGGCTACACACGTGCTACAATGGTTGGTACAACGAG TCGCAAGCCGGTGACGGCAAGCTAATCTCTTAAAGCCAATCTCAGTTCCGAT TGTAAGGCTGCAACTCGCCTACATGAAGTCGGAATCGCTAGTAATCGCGGATC AGCACGCCGCGGTGAATACGTTCCCGGGCCTTGTAACACCGCCCGTCACAC CACGAGAGTTTGTAACACCCGAAGTCGGTGAGGTAACCATTTGGAGCCAGC CGCCTAAGGTGGGATAGATGATT
Clostridium _sp_CL_6	SEQ ID NO: 287	GCGATGAAGCCCTTCGGGGTGGATTAGCGGCGGACGGGTGAGTAACACGTG GGTAACCTGCCTTGTAAGAGGGGGATAGCCTTCCGAAAGGAAGATTAATACC GCATAACATCTTTTTATCGCATGGTAGAAAGATCAAAGGAGCAATCCGCTAC AAGATGGACCCGCGGCGCATTAGCTAGTTGGTGAGGTAACGGCTCACCAAG GCGACGATGCGTAGCCGACCTGAGAGGGTGATCGGCCACATTGGAAGTGAAG ACACGGTCCAGACTCCTACGGGAGGCAGCAGTGGGGAATATTGCGCAATGG GGGAAACCCTGACGCAGCAACGCCGCGTGAATGAAGAAGGCCCTTAGGGTTG TAAAGTTCTGTTTACGGGGACGATAATGACGGTACCCGTGGGGAAGCCACG GCTAACTACGTGCCAGCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCC GGATTTACTGGGCGTAAAGGATGCGTAGGCGGATGTTTAAAGTGAGATGTGA AATACCCGGGCTCAACTTGGGTGCTGCATTTCAAAGTGGACATCTAGAGTGC GGGAGAGGAAAGCGGAATTCCTAGTGTAGCGGTGAAATGCGTAGAGATTAG GAAGAACACCAAGTGGCGAAGGCGGCTTTCTGGACCGTAAGTACGCTGAGG CATGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCG TAAACGATGAATACTAGGTGTAGGAGGTATCGACTCCTTCTGTGCCGCAGTT AACACAATAAGTATTCGCCTGGGAAGTACGATCGCAAGATTAAGAACTCAA AGGAATTGACGGGGGCGCCGACAAGCAGCGGAGCATGTGGTTAATTCGAA GCAACGCGAAGAACCTTACCTAGACTTGACATCTCCTGAATTACTCTTAATC GAGGAAGCCCTTCGGGGCAGGAAGACAGGTGGTGCATGGTTGTCTGTCAGCT CGTGTCTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTTATCATT AGTTGCTACCATTAAGTTGAGCACTCTAGTGAGACTGCCACGGTTAACGTGG AGGAAGGTGGGGATGACGTCAAATCATCATGCCCTTATGTCTAGGGCTAC ACACGTGCTACAATGGTGAATACAAATAGATGCAATACCGCGAGGTGGAGC CAAATAATAAATTCATCCAGTTCGGATTGCAGTCTGAACTCGACTGCAT GAAGCCGAGTTGCTAGTAATCGCGAATCAGCATGTGCGGGTGAATACGTT CCCGGGCCTTGTAACACCGCCCGTCACACCATGAGAGCTGGTAACACCCG AAGTTCCGTGAGGTAACCGCA

Table 6 – Full length 16S rDNA sequences of species (positive correlates)

Species Name	Sequence Identifier	16S sequence
Streptococcus_mitis_o ralis_pneumoniae	SEQ ID NO: 288	aatgagagtttgatcctggctcaggacgaacgctggcggcgtgcctaatacatgcaagtagaacgctgaaggagga gcttgcttctctgtagatgagttgcgaacgggtgagtaacgcgtaggtaacctgcctgtagcgggggataactattgga aacgataagctaataccgcataagagtgatgttgcacattgcttaaaagggtgcacttgcactactaccagatgga cctgcgttgattagctagttggtgggtaacggctcaccaggcgacgatacatagccgacctgagagggtgatcg gccacactgggactgagacacggccagactcctacgggagcgacgtagggaatcttcgcaatggacggaa gtctgaccgagcaacgccgcgtgagtgaaagggttttcggatcgtaaagctctgttgtaagaagaacgagtggtg agagtggaaagttcacactgtgacggtaattaccagaaaggacggcctaactacgtgccagcagccgcggttaata cgtaggtcccagcgtgttcggatttattggcgtaaaagcagcgcagcgggttagataagtcgaagttaaaggct gtggcctaaccatagtaggctttgaaactgttaactgagtgcaagaggggagagtggaattccatgtgtagcgggtg aaatgcgtagatatatggaggaaacaccgggtggcgaaagcggctctctgctgtgaactgacgctgaggtcgaaag cgtggggagcaaacaggattagataccctggtagtcacgctgtaaacgatgagtgctaggtgttagacccttccgg ggttagtgccgtagctaacgcattaaagcactccgcctgggagtagcaccgcaaggttgaaactcaaaaggaattga cggggggccgcacaagcgggtggagcatgtggttaattcgaagcaacgcgaagaacctaccaggtcttgacatcc ctctgaccgctctagatagatagtttcttcgggacagaggtgacaggtggtgcatggtgtcgtcagctcgtgtcgt gagatgttgggttaagtcgccgaacgagcgaacccctattgttagttgccatcattcagttgggcactctagcgagac tggcggtaataaaccggagggaaggtggggatgacgtcaatcatcatgcccccttatgacctgggctacacacgtgct acaatggctggtacaacgagtcgaagccgggtgacggcaagcctaactcttaaaagccagtcacgttcggatttagg ctgcaactcgcctacatgaagtcggaatcgtagtaatcgcggatcagcagccgcgggtgaatacgttcccgccct tgtacacaccgcccgctcacaccagagagtttgaacaccggaagtcggtaggtaaccgtaaggagccagccgcc taaggtgggatagatgattgggtgaagtcgtaacaaggtagccgtatcgggaaggtgcggctggatcacctcctt
Streptococcus_mitis_o ralis_pneumoniae	SEQ ID NO: 289	aatgagagtttgatcctggctcaggacgaacgctggcggcgtgcctaatacatgcaagtagaacgctgaaggagga gcttgcttctctgtagatgagttgcgaacgggtgagtaacgcgtaggtaacctgcctgtagcgggggataactattgga aacgataagctaataccgcataagagtgatgttgcacattgcttaaaagggtgcacttgcactactaccagatgga cctgcgttgattagctagttggtgggtaacggctcaccaggcgacgatacatagccgacctgagagggtgatcg gccacactgggactgagacacggccagactcctacgggagcgacgtagggaatcttcgcaatggacggaa gtctgaccgagcaacgccgcgtgagtgaaagggttttcggatcgtaaagctctgttgtaagaagaacgagtggtg agagtggaaagttcacactgtgacggtaattaccagaaaggacggcctaactacgtgccagcagccgcggttaata cgtaggtcccagcgtgttcggatttattggcgtaaaagcagcgcagcgggttagataagtcgaagttaaaggct gtggcctaaccatagtaggctttgaaactgttaactgagtgcaagaggggagagtggaattccatgtgtagcgggtg aaatgcgtagatatatggaggaaacaccgggtggcgaaagcggctctctgctgtgaactgacgctgaggtcgaaag cgtggggagcaaacaggattagataccctggtagtcacgctgtaaacgatgagtgctaggtgttagacccttccgg ggttagtgccgtagctaacgcattaaagcactccgcctgggagtagcaccgcaaggttgaaactcaaaaggaattga cggggggccgcacaagcgggtggagcatgtggttaattcgaagcaacgcgaagaacctaccaggtcttgacatcc ctctgaccgctctagatagatagtttcttcgggacagaggtgacaggtggtgcatggtgtcgtcagctcgtgtcgt gagatgttgggttaagtcgccgaacgagcgaacccctattgttagttgccatcattcagttgggcactctagcgagac tggcggtaataaaccggagggaaggtggggatgacgtcaatcatcatgcccccttatgacctgggctacacacgtgct acaatggctggtacaacgagtcgaagccgggtgacggcaagcctaactcttaaaagccagtcacgttcggatttagg ctgcaactcgcctacatgaagtcggaatcgtagtaatcgcggatcagcagccgcgggtgaatacgttcccgccct tgtacacaccgcccgctcacaccagagagtttgaacaccggaagtcggtaggtaaccgtaaggagccagccgcc taaggtgggatagatgattgggtgaagtcgtaacaaggtagccgtatcgggaaggtgcggctggatcacctcctt
Streptococcus_mitis_o ralis_pneumoniae	SEQ ID NO: 290	aatgagagtttgatcctggctcaggacgaacgctggcggcgtgcctaatacatgcaagtagaacgctgaaggagga gcttgcttctctgtagatgagttgcgaacgggtgagtaacgcgtaggtaacctgcctgtagcgggggataactattgga aacgataagctaataccgcataagagtgatgttgcacattgcttaaaagggtgcacttgcactactaccagatgga cctgcgttgattagctagttggtgggtaacggctcaccaggcgacgatacatagccgacctgagagggtgatcg gccacactgggactgagacacggccagactcctacgggagcgacgtagggaatcttcgcaatggacggaa gtctgaccgagcaacgccgcgtgagtgaaagggttttcggatcgtaaagctctgttgtaagaagaacgagtggtg agagtggaaagttcacactgtgacggtaattaccagaaaggacggcctaactacgtgccagcagccgcggttaata cgtaggtcccagcgtgttcggatttattggcgtaaaagcagcgcagcgggttagataagtcgaagttaaaggct gtggcctaaccatagtaggctttgaaactgttaactgagtgcaagaggggagagtggaattccatgtgtagcgggtg aaatgcgtagatatatggaggaaacaccgggtggcgaaagcggctctctgctgtgaactgacgctgaggtcgaaag cgtggggagcaaacaggattagataccctggtagtcacgctgtaaacgatgagtgctaggtgttagacccttccgg ggttagtgccgtagctaacgcattaaagcactccgcctgggagtagcaccgcaaggttgaaactcaaaaggaattga cggggggccgcacaagcgggtggagcatgtggttaattcgaagcaacgcgaagaacctaccaggtcttgacatcc ctctgaccgctctagatagatagtttcttcgggacagaggtgacaggtggtgcatggtgtcgtcagctcgtgtcgt gagatgttgggttaagtcgccgaacgagcgaacccctattgttagttgccatcattcagttgggcactctagcgagac tggcggtaataaaccggagggaaggtggggatgacgtcaatcatcatgcccccttatgacctgggctacacacgtgct acaatggctggtacaacgagtcgaagccgggtgacggcaagcctaactcttaaaagccagtcacgttcggatttagg ctgcaactcgcctacatgaagtcggaatcgtagtaatcgcggatcagcagccgcgggtgaatacgttcccgccct tgtacacaccgcccgctcacaccagagagtttgaacaccggaagtcggtaggtaaccgtaaggagccagccgcc taaggtgggatagatgattgggtgaagtcgtaacaaggtagccgtatcgggaaggtgcggctggatcacctcctt

		cgtaggtcccgagcgttgcggatttattggcgtaaaagcagcgcagcgcggttagataagctgaagttaaaggct gtggcctaaccatagtaggctttgaaactgttaacttgagtgcagaggaggagagtggaattccatgtgtagcgggtg aatgcgtagatatatggaggaaacaccgggtggcgaaagcggctctctgctgttaactgacgctgaggtcgaag cgtggggagcaaacaggattagataccctggtagtcacgctgtaaacgatgagtgtgaggtgttagaccctttccgg ggtttagtgccgtagctaaccgacttaagcactccgctggggagtagaccgcaaggtgaaactcaaaaggaattga cggggggccgcacaagcgtggagcatgtgtttaattcgaagcaacgcgaagaacctaccaggtcttgacatcc ctctgaccgctctagatagatagtttcttcgggacagaggtgacaggtggtgcatggtgtcgtcagctcgtgtcgt gagatgttgggttaagtcccgcaacgagcgcgaacccctattgttagttgccatcattcagttgggcaactctagcgagac tgccggtataaaaccggaggaaaggtgggatgacgtcaaatcatcatgcccttatgacctgggctacacacgtgct acaatggctggtacaacgagtcgaagcgggtgacggcaagctaattcttaaaagccagtcacgttcggatttagg ctgcaactcgcctacatgaagtcggaatcgtagtaatcgcggatcagcagccggcggtgaatacgttccggggcct tgtacacaccgccgacacacgagagtttgaacaccggaagtcggtaggtaaccgtaaggagccagccgcc taaggtgggatagatgattgggggtaagtcgtaacaaggtagccgtatcggaaggtgcggctggtatcacctcctt
Streptococcus_mitis_o ralis_pneumoniae	SEQ ID NO: 291	aatgagagtttgatcctggctcaggacgaacgctggcgccgtgcctaatacatgcaagtagaacgctgaaggagga gcttgcttctctgatgagttgcgaacgggtgagtaacgcgtagtaacctgctgtgagcggggataactattgga aacgataagcataaccgcataagagtggtgatgttgcattgacattgcttaaaagggtgcattgcatcactaccagatgga cctgctgttattagctagttgtgggtaacggctcaccgaagggcagcagatacatagccgacctgagaggggtgacg gccacactgggactgagacacggccagactcctacgggagggcagcagtagggaatcttcgcaatggcaggaa gtctgaccgagcaacggcgctgagtggaagaggttttcggatcgtaaaagctctgttgaagagaagaacgagtggtg agagtggaaagttcacactgtgacggtagtttaccagaaaggagcggtaactactgtgccagcagccgcggtaata cgtaggtcccagcgtgtccgatttattggcgtaaaagcagcgcagcgcggttagataagctgaagttaaaggct gtggcctaaccatagtaggctttgaaactgttaacttgagtgcagaggaggagagtggaattccatgtgtagcgggtg aatgcgtagatatatggaggaaacaccgggtggcgaaagcggctctctgctgtgtaactgacgctgaggtcgaag cgtggggagcaaacaggattagataccctggtagtcacgctgtaaacgatgagtgtgaggtgttagaccctttccgg ggtttagtgccgtagctaaccgacttaagcactccgctggggagtagaccgcaaggtgaaactcaaaaggaattga cggggggccgcacaagcgtggagcatgtgtttaattcgaagcaacgcgaagaacctaccaggtcttgacatcc ctctgaccgctctagatagatagtttcttcgggacagaggtgacaggtggtgcatggtgtcgtcagctcgtgtcgt gagatgttgggttaagtcccgcaacgagcgcgaacccctattgttagttgccatcattcagttgggcaactctagcgagac tgccggtataaaaccggaggaaaggtgggatgacgtcaaatcatcatgcccttatgacctgggctacacacgtgct acaatggctggtacaacgagtcgaagcgggtgacggcaagctaattcttaaaagccagtcacgttcggatttagg ctgcaactcgcctacatgaagtcggaatcgtagtaatcgcggatcagcagccggcggtgaatacgttccggggcct tgtacacaccgccgacacacgagagtttgaacaccggaagtcggtaggtaaccgtaaggagccagccgcc taaggtgggatagatgattgggggtaagtcgtaacaaggtagccgtatcggaaggtgcggctggtatcacctcctt
Proteus_mirabilis	SEQ ID NO: 292	ttgaagagtttgatcatggctcagattgaacgctggcgagcgcctaacacatgcaagtcgagcggtaacaggagaa agcttgcttcttctgctgacgagcggcgagcgggtgagtaattgtatggggatctgcccagatagagggggataactact ggaaacggtggtataaccgcataatgtctacggaccaaaagcaggggctcttcggaccttgcaactatcgatgaacc catatgggattagctagtaggtgggttaaaggctcacttagggcagcatctctagctggtctgagaggtatgacgc cacactgggactgagacacggccagactcctacgggagggcagcagtggggaattatgcacaatgggcgaagc ctgatgcagccatccgctgtatgaagaaggccttaggggttgaagtactttcagcggggaggaaggtgataagg ttaataccctgtcaattgacgttaccgcagaagaagcaccggcctaactcctgcccagcagccgcggtataacgga gggtgcaagcgtaatcggaattactggcgtaaaagcgacgcagggcgtcaattaagtcagatgtgaaagccccg agcttaacttgggaattgcatctgaaactggttgctagagttctgtagagggggtagaattccatgtgacgggtga aatgcgtagagatgtggaggaataaccggtggcggaagcggccccctggacaaagactgacgctcaggtgcgaaa gcgtggggagcaaacaggattagataccctggtagtccacgctgtaaacgatgtcgattagaggttgtgtcttgaa ccgtggctcttgagagtaacgcgttaaatcagccgctggggagtagcggcgcaaggttaaaactcaaatgaattga cggggggccgcacaagcgtggagcatgtgtttaattcgtatgaacgcgaagaaactacactcttgacatcca gcgaatccttagatagatagaggtgcttcgggaacgctgagacaggtgtctgcatggtgtcgtcagctcgtgtgt gaaatgttgggttaagtcccgcaacgagcgaacccctatccttgggtgccaagcagtaaatgttgggaactcaaggga gactgccggtgataaaccggaggaaggtggggatgacgtcaagtcacatggtgcccttaccagtagggctacacacg tgtacaatggcagatacaaaagagaagcgacctcgcgagagcaagcggaactcataaagctgtcgtagtccggatt ggagctgcaactcactccatgaagtcggaatcgtagtaatcgtatcagaatgtctacgggtgaatacgttccgg

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DEMANDE OU BREVET VOLUMINEUX

LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVET COMPREND PLUS D'UN TOME.

CECI EST LE TOME 1 DE 2
CONTENANT LES PAGES 1 À 164

NOTE : Pour les tomes additionels, veuillez contacter le Bureau canadien des brevets

JUMBO APPLICATIONS/PATENTS

THIS SECTION OF THE APPLICATION/PATENT CONTAINS MORE THAN ONE VOLUME

THIS IS VOLUME 1 OF 2
CONTAINING PAGES 1 TO 164

NOTE: For additional volumes, please contact the Canadian Patent Office

NOM DU FICHIER / FILE NAME :

NOTE POUR LE TOME / VOLUME NOTE:

What is claimed is:

1. A composition comprising a therapeutically effective amount of a microbiome composition comprising spore-former bacteria for use in reducing the abundance of at least one antibiotic-resistance gene in the microbiome of a subject.
2. A composition comprising a therapeutically effective amount of a microbiome composition comprising *Firmicutes* derived from one or more healthy human subjects for use in treating a subject at risk for or diagnosed with an undesirable level or population of drug-resistant bacteria.
3. A ROAR composition for use reducing the abundance of antibiotic-resistant bacteria in the microbiome of a subject.
4. A ROAR composition for use in disrupting a transmission cycle of antibiotic resistance genes, by administration to at least two individuals with a high likelihood of direct or indirect contact, including optionally contact with a bodily fluid or waste.
5. The composition for use of claim 1 or 4, wherein the antibiotic-resistance gene(s) is selected from those listed in Table 1.
6. The composition for use of claim 5, wherein the abundance of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more antibiotic resistance genes of Table 1 is reduced.
7. The composition for use of any one of claims 1 to 6, wherein the drug or antibiotic is from a drug class listed in Table 2.
8. The composition for use of any one of claims 1 to 7, further comprising reducing the abundance of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, or more antibiotic resistant genes or drug classes.
9. The composition for use of any one of claims 1 to 8, wherein the composition comprises one or more species listed in Table 3.
10. The composition for use of claim 9, wherein the composition comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more species listed in Table 3.

11. The composition for use of claim 9 or 10, wherein the species comprises a 16S rDNA, V4, and/or V6 sequence listed in Table 5, or a sequence having at least 95% identity thereto.
12. The composition for use of any one of claims 1 to 11, wherein the composition does not comprise one or more species of Table 4.
13. The composition for use of any one of claims 1 to 12, wherein the bacteria in the composition are in the form of spores.
14. The composition for use of any one of claims 1 to 13, wherein the bacteria in the composition are cultured.
15. The composition for use of any one of claims 1 to 14, wherein the composition comprises *Firmicutes* and one or more species of *Bacteroides*.
16. The composition for use of any one of claims 1 to 15, wherein the subject has a reduced abundance of one, two, or three bacteria selected from the group consisting of quinolone resistant, beta-lactam-resistant, and carbapenem-resistant bacteria.
17. The composition for use of any one of claims 1 to 16, wherein the subject has been unresponsive to antibiotic treatment.
18. The composition for use of any one of claims 1 to 17, wherein the subject has or is at risk of developing *C. difficile* infection.
19. The composition for use of any one of claims 1 to 18, wherein the subject has or is at risk of developing a colitis.
20. The composition for use of claim 19, wherein the colitis is Crohn's disease or ulcerative colitis.
21. The composition for use of claim 20, wherein the colitis is mild to moderate ulcerative colitis.

22. A composition comprising bacterial species associated with a decrease in the abundance of at least one antibiotic resistance gene in a subject.
23. The composition of claim 22, wherein the antibiotic-resistance gene(s) is selected from those listed in Table 1.
24. The composition of claim 23, wherein the abundance of at least 2, 3, 4, 5, 6, 7, 8, 9, 10, or more antibiotic resistance genes of Table 1 is decreased.
25. The composition of any one of claims 22 to 24, wherein the antibiotic is from a class listed in Table 2.
26. The composition of any one of claims 22 to 25, wherein the composition comprises one or more species listed in Table 3.
27. The composition of claim 26, wherein the composition comprises 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or more species listed in Table 3.
28. The composition of any one of claims 20 to 27, wherein the species comprises a 16S rDNA, V4, and/or V6 sequence listed in Table 5, or a sequence having at least 95% identity thereto.
29. The composition of any one of claims 22 to 28, wherein the composition does not comprise one or more species of Table 4, and optionally the one or more species of Table 4 comprises a 16S rDNA, V4, and/or V6 sequence listed in Table 6, or a sequence having at least 95% identity thereto.
30. The composition of any one of claims 22 to 29, wherein the composition comprises one or more species of *Firmicutes*.
31. The composition of any one of claims 22 to 30, wherein the composition comprises one or more species of *Bacteroides*.
32. The composition of any one of claims 22 to 31, wherein the bacteria in the composition are in the form of spores.

33. The composition of any one of claims 22 to 32, wherein the bacteria in the composition are obtained from a spore fraction derived from feces of a healthy subject.
34. The composition of any one of claims 22 to 33, wherein the composition comprises one or more species of *Firmicutes*, and optionally one or more species of *Bacteroides*, as the sole bacterial species in the composition.
35. The composition of any one of claims 22 to 34, wherein the bacteria in the composition are cultured.
36. A composition comprising bacteria for use in decreasing the abundance of at least one antibiotic resistance gene in a subject to whom the composition is administered.
37. The composition of claim 36, wherein the bacteria in the composition are obtained from a spore fraction derived from feces of a healthy subject.
38. The composition of claim 36, wherein the bacteria are selected for being associated with a decrease in the abundance of one or more antibiotic resistance genes in a subject.
39. The composition of any one of claims 36 to 38, wherein the antibiotic resistance gene(s) is selected from Table 1.
40. The composition of any one of claims 36 to 39, wherein the composition comprises at least one species selected from Table 3.
41. The composition of any one of claims 36 to 40, wherein the composition does not comprise a species of Table 4.
42. A method for identifying a bacterial composition useful for decreasing the abundance of at least one antibiotic resistance gene in a subject to whom the composition is administered, the method comprising screening the composition for the presence of one or more bacterial species of Table 3, wherein detection of one or more species of Table 3 in the bacterial composition indicates the identification of a composition that can be used to decrease the abundance of at least one antibiotic resistance gene.

43. The method of claim 42, wherein the screening comprises detection of one or more 16S rDNA, V4, and/or V6 sequences of Table 5 in the composition, or a sequence having at least 95% identity thereto.

44. The method of claim 42 or 43, further comprising screening for the presence of one or more bacterial species of Table 4, and optionally the one or more species of Table 4 comprises a 16S rDNA, V4, and/or V6 sequence listed in Table 6, or a sequence having at least 95% identity thereto.

45. A method for screening a potential donor of feces for use in therapeutic methods, the method comprising testing a feces sample from the potential donor for the presence of a bacterial species from Table 3 or Table 4, wherein detection of one or more species from Table 3 indicates that the potential donor may proceed to become a donor, while detection of one or more species from Table 4 indicates that the potential donor should not become a donor.

46. A method for determining whether a subject could benefit from treatment with a ROAR composition, the method comprising determining whether a sample from the subject comprises one or more species of bacteria from Table 4, wherein detection of one or more species from Table 4 indicates that the subject may benefit from treatment with a ROAR composition.

47. A composition of any one of claims 22 to 41, wherein the bacteria in the composition demonstrate the ability to decrease the abundance of one or more antibiotic resistance gene(s) in an animal model of antibiotic resistance.

48. The composition of claim 47, wherein the animal model comprises a mouse treated with one or more antibiotics and then colonized with one or more bacteria harboring one or more antibiotic resistance genes.

49. The composition of claim 48, wherein the mice are colonized with vancomycin resistant bacteria or vancomycin and carbapenem resistant bacteria.

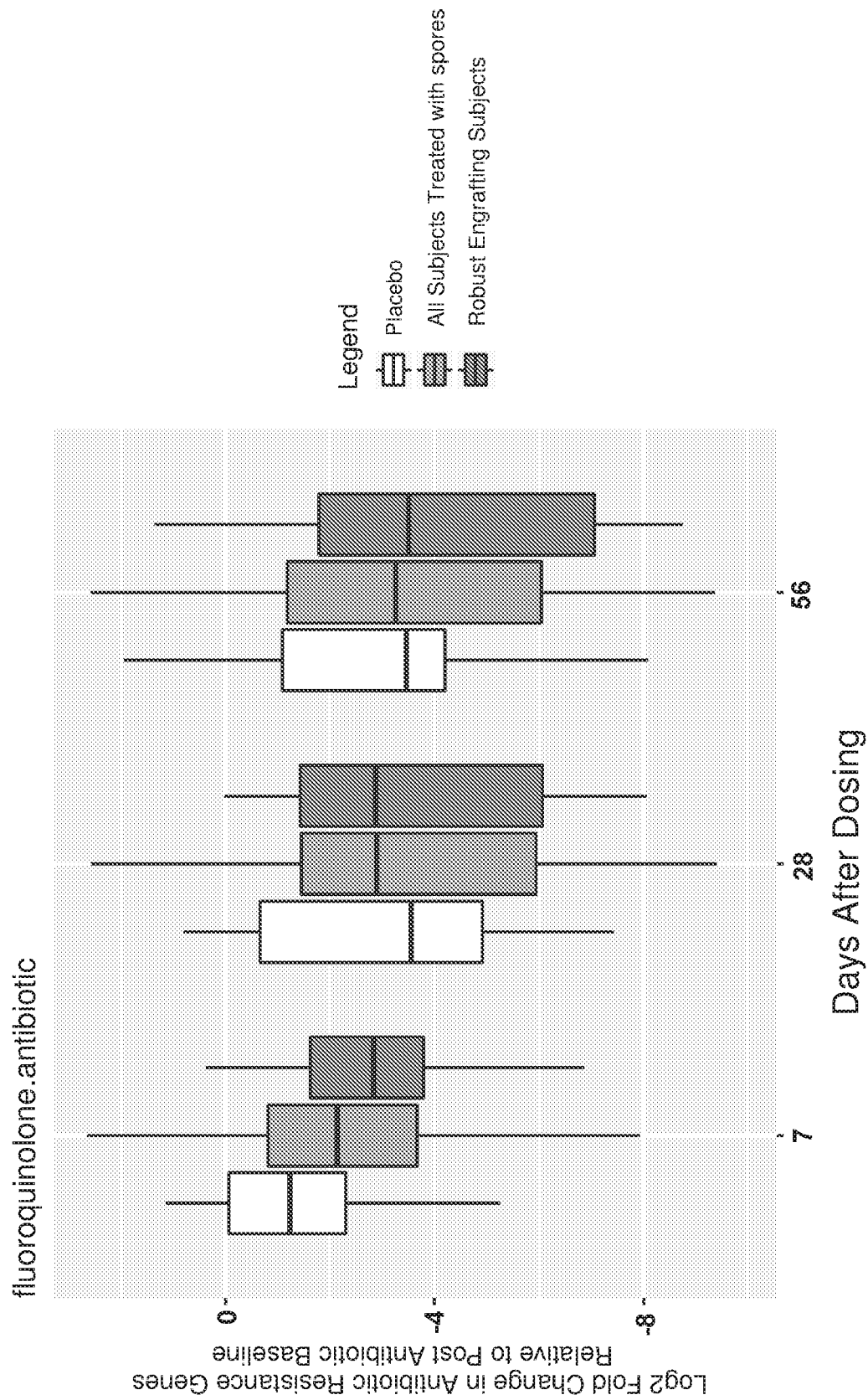


FIG. 1

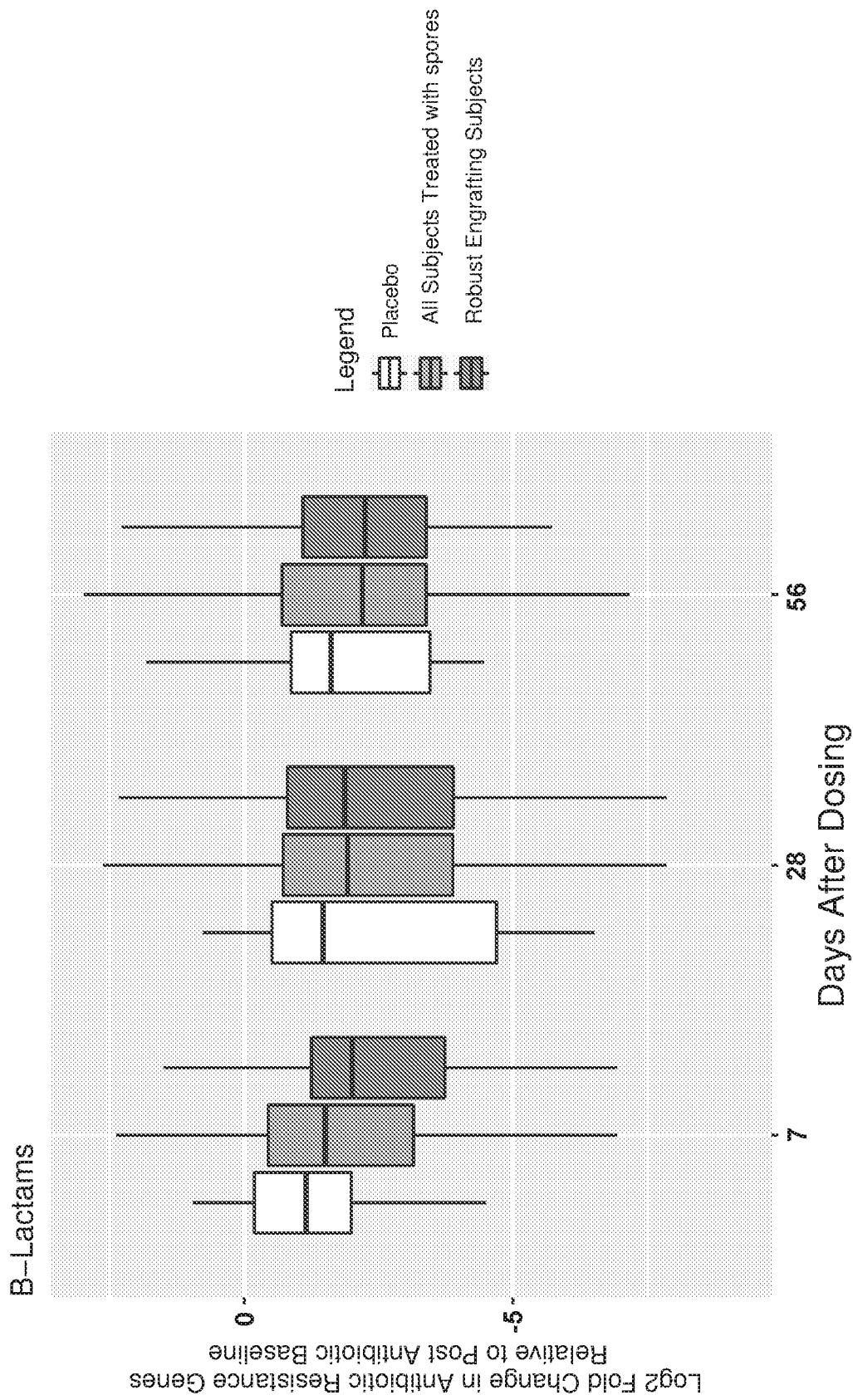
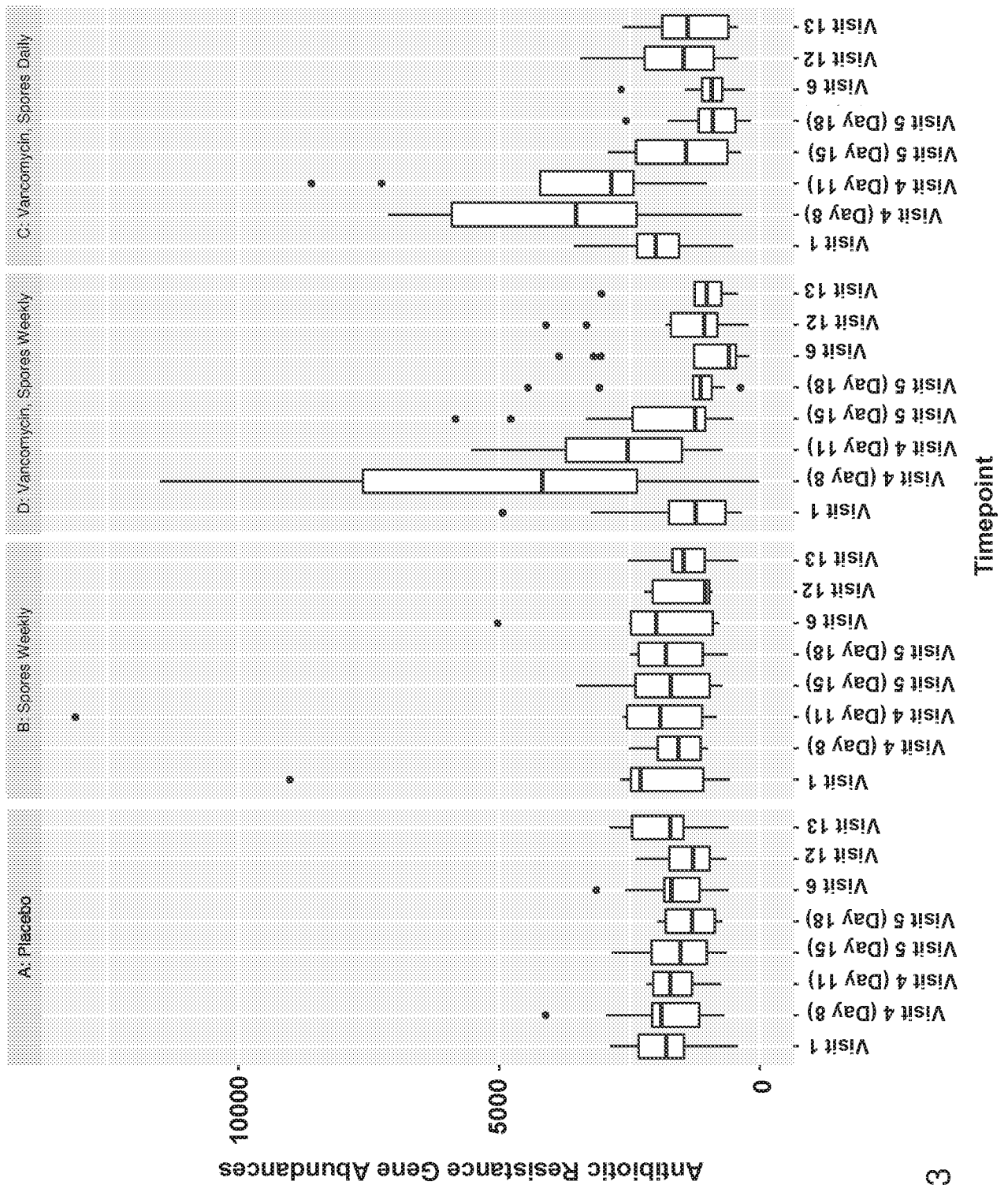


FIG. 2



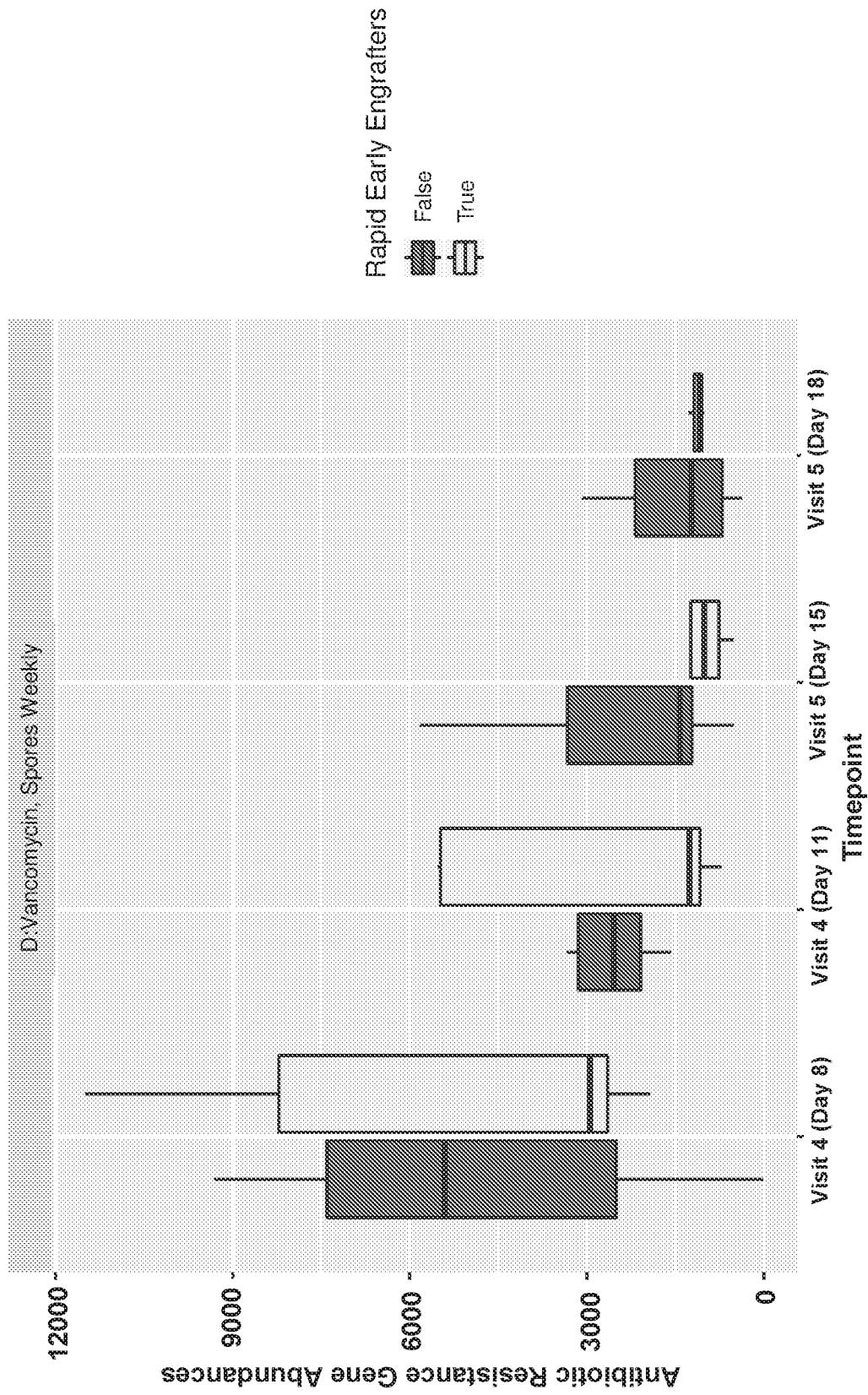


FIG. 4