non-viable organisms leading to possible false-positive results and also cannot determine if the detected gene is expressed and are thus incapable of measuring the phenotypic response of an organism to an antimicrobial and thus cannot generally determine antimicrobial susceptibility.

Due to these limitations, there is a need for a means of determining the underlying mechanism that imparts a phenotypic response in an organism. The identity of the specific underlying mechanism that imparts a given phenotype can be important information for epidemiological analysis and other related analyses.

SUMMARY

In one aspect, the present invention refers to a method for determining a mechanism for an antimicrobial susceptibility phenotype, comprising: providing a sample comprising at least one microorganism that is not susceptible to at least one antimicrobial agent; contacting the sample with the antimicrobial agent, wherein the antimicrobial agent can kill, inhibit the growth, or otherwise compromise the viability of one or more microorganisms; contacting the at least one microorganism with at least one compound comprising an oligonucleotide molecule targeting at least one specific nucleic acid sequence that is involved in a non-susceptible phenotype to the antimicrobial agent; contacting the sample with a non-replicative transduction particle (NRTP) comprising a reporter nucleic acid molecule encoding a reporter molecule under conditions such that the NRTP inserts the reporter nucleic acid molecule encoding the reporter molecule into the microorganism and such that the reporter molecule provides the detectable indication of viability; and determining the mechanism for the antimicrobial susceptibility phenotype of the microorganism based on the presence or absence of a detectable indication of viability associated with the microorganism when the microorganism is in contact with the antimicrobial agent and the oligonucleotide molecule, wherein the presence of the detectable indication of viability indicates that the specific nucleic acid sequence targeted by the oligonucleotide molecule is not related to the mechanism for the antimicrobial susceptibility phenotype to the antimicrobial agent, and wherein the absence of the detectable indication of viability indicates that the specific nucleic acid sequence targeted by the oligonucleotide molecule is related to the mechanism for the antimicrobial susceptibility phenotype to the antimicrobial agent. In another aspect, the present invention refers to a method for determining a mechanism involved in a non-susceptible phenotype, comprising: providing a sample comprising an Enterobacteriaceae that is not susceptible to carbapenem; contacting the sample with carbapenem; contacting the Enterobacteriaceae with an oligonucleotide molecule that targets a gene associated with carbapenem resistance; contacting the sample with a non-replicative transduction particle (NRTP) comprising a reporter nucleic acid molecule encoding a reporter molecule under conditions such that the NRTP inserts the reporter nucleic acid molecule encoding the reporter molecule into the Enterobacteriaceae and such that the reporter molecule provides the detectable indication of viability; and determining the mechanism of carbapenem non-susceptibility for the Enterobacteriaceae based on the presence or

absence of a detectable indication of viability associated with the Enterobacteriaceae when the Enterobacteriaceae is in contact with the carbapenem and the oligonucleotide molecule, wherein the presence of the detectable indication of viability indicates that the gene targeted by the oligonucleotide molecule is not related to the mechanism of carbapenem non-susceptibility, and wherein the absence of the detectable indication of viability indicates that the gene targeted by the oligonucleotide molecule is related to the mechanism of carbapenem non-susceptibility. In yet another aspect, the present invention refers to a method for reducing the amount of potentially cross-reactive or interfering organisms in an assay designed to detect a detectable indication of viability of a target organism comprising: providing a sample comprising at least one organism that is potentially cross-reactive or interfering in said assay designed to detect the detectable indication of viability of the target organism; contacting the cross-reactive or interfering organism with at least one compound comprising an oligonucleotide molecule targeting at least one specific nucleic acid sequence that is involved with the viability of the potentially cross-reactive or interfering organism, wherein the specific nucleic acid sequence is unique to the potentially cross-reactive or interfering organism; and causing the potentially cross-reactive or interfering organism to lose viability; and contacting the sample with a non-replicative transduction particle (NRTP) comprising a reporter nucleic acid molecule encoding a reporter molecule under conditions such that the NRTP inserts the reporter nucleic acid molecule encoding the reporter molecule into the target organism and such that the reporter molecule provides a detectable indication of viability of the target organism. Disclosed herein is a method for determining a mechanism for an antimicrobial susceptibility phenotype, comprising: providing a sample comprising at least one microorganism that is not susceptible to at least one antimicrobial agent; contacting the sample with the antimicrobial agent, wherein the antimicrobial agent can kill, inhibit the growth, or otherwise compromise the viability of one or more microorganisms; contacting the sample with at least one compound comprising an oligonucleotide molecule targeting at least one specific nucleic acid sequence that is involved in a non-susceptible phenotype to the antimicrobial agent, optionally wherein the oligonucleotide molecule inhibits the nucleic acid sequence; and determining the mechanism for the antimicrobial susceptibility phenotype of the microorganism based on the presence or absence of a detectable indication of viability associated with the microorganism when the microorganism is in contact with the antimicrobial agent and the oligonucleotide molecule, wherein the presence of the detectable indication of viability indicates that the specific nucleic acid sequence targeted by the oligonucleotide molecule is not related to the mechanism for the antimicrobial susceptibility phenotype to the antimicrobial agent, and wherein the absence of the detectable indication of viability indicates that the specific nucleic acid sequence targeted by the oligonucleotide molecule is related to the mechanism for the antimicrobial susceptibility phenotype to the antimicrobial agent. Other methods, compositions, systems, cultures, molecules, and kits are similarly described herein.

Also disclosed herein is a method for determining a mechanism for an antimicrobial susceptibility phenotype comprising: providing a sample comprising at least one microorganism that is not susceptible to at least one antimicrobial agent, and wherein the sample further comprises: the antimicrobial agent, wherein the antimicrobial agent can kill, inhibit the growth, or otherwise compromise the viability of one or more microorganisms; and at least one compound comprising an oligonucleotide molecule targeting at least one specific nucleic acid sequence that is involved with a non-susceptible phenotype to the antimicrobial agent; and determining the mechanism for the non-susceptible phenotype for the microorganism based on the presence or absence of a detectable indication of viability associated with the microorganism when the microorganism is in contact with the antimicrobial agent and the oligonucleotide molecule, wherein the presence of the detectable indication of viability indicates that the specific nucleic acid sequence targeted by the oligonucleotide molecule is not related to the mechanism for the non-susceptible phenotype to the antimicrobial agent, and wherein the absence of the detectable indication of viability indicates that the specific nucleic acid sequence targeted by the oligonucleotide molecule is related to the mechanism for the non-susceptible phenotype to the antimicrobial agent.

In some examples, the compound is a PNA (peptide nucleic acid). In some examples, the compound is a peptide-PNA. In some examples, the peptide facilitates uptake of the oligonucleotide molecule into the microorganism. In some examples, the PNA targets a translation initiation region (TIR) of a gene. In some examples, the peptide-PNA targets a β-lactam resistance gene or a vancomycin resistance gene. In some examples, the peptide – PNA targets a bla_{KPC-3} gene, a bla_{NDM-1} gene, a bla_{SHV-18} gene, a *vanC* gene.

Also disclosed herein is a method for determining a mechanism involved in a non-susceptible phenotype, comprising: providing a sample comprising an Enterobacteriaceae that is not susceptible to carbapenem; contacting the sample with carbapenem; contacting the sample with an oligonucleotide molecule that targets a gene associated with carbapenem resistance; and determining the mechanism of carbapenem non-susceptibility for the Enterobacteriaceae based on the presence or absence of a detectable indication of viability associated with the Enterobacteriaceae when the Enterobacteriaceae is in contact with the carbapenem and the oligonucleotide molecule, wherein the presence of the detectable indication of viability indicates that the gene targeted by the oligonucleotide molecule is not related to the mechanism of carbapenem non-susceptibility, and wherein the absence of the detectable indication of viability indicates that the gene targeted by the oligonucleotide molecule is related to the mechanism of carbapenem non-susceptibility.

Also disclosed herein is a method for determining the presence of an organism of interest, comprising: providing a sample potentially comprising at least one organism of interest; contacting the sample with at least one compound comprising an oligonucleotide molecule targeting at least one specific nucleic acid sequence that is involved with the viability of the organism, wherein the

specific nucleic acid sequence is unique to the organism; and determining the presence of the organism based on the presence or absence of a detectable indication of viability associated with the organism when the organism is in contact with the oligonucleotide molecule, wherein the presence of the detectable indication of viability indicates that the organism of interest is not present in the sample, and wherein the absence of the detectable indication of viability indicates that the organism of interest may be present in the sample

Also disclosed herein is a method for determining a presence of an organism of interest, comprising: providing a sample potentially comprising at least one organism of interest and at least one compound comprising an oligonucleotide molecule targeting at least one specific nucleic acid sequence that is involved with the viability of the organism, wherein the specific nucleic acid sequence is unique to the organism; and determining the presence of the organism based on the presence or absence of a detectable indication of viability associated with the organism when the organism is in contact with the oligonucleotide molecule, wherein the presence of the detectable indication of viability indicates that the organism of interest is not present in the sample, and wherein the absence of the detectable indication of viability indicates that the organism of interest may be present in the sample.

Also disclosed herein is a method for determining the presence of an organism of interest, comprising: providing a sample potentially comprising at least one organism of interest; contacting a first portion of the sample with at least one compound comprising an oligonucleotide molecule targeting at least one specific nucleic acid sequence that is involved with the viability of the organism, wherein the specific nucleic acid sequence is unique to the organism; determining the presence of the organism in the first portion of the sample based on the presence or absence of a detectable indication of viability associated with the organism when the organism is in contact with the oligonucleotide molecule, wherein the presence of the detectable indication of viability indicates that the organism of interest is not present in the sample, and wherein the absence of the detectable indication of viability indicates that the organism of interest may be present in the sample; and determining the presence of the organism in a second portion of the sample that does not contain the oligonucleotide molecule based on the presence or absence of a detectable indication of viability associated with the organism, wherein the presence of the detectable indication of viability indicates that the organism of interest may be present in the sample, and wherein the absence of the detectable indication of viability indicates that the organism of interest is not present in the sample.

Also disclosed herein is a method for reducing the amount of potentially cross-reactive or interfering organisms in an assay designed to detect a target organism, comprising: obtaining a sample potentially comprising at least one organism that is potentially cross-reactive or interfering in an assay designed to detect a target organism; contacting the cross-reactive or interfering organism with at least one compound comprising an oligonucleotide molecule targeting at least one specific nucleic

acid sequence that is involved with the viability of the potentially cross-reactive or interfering organism, wherein the specific nucleic acid sequence is unique to the organism; and causing the organism to lose viability. In some examplesthe at least one specific nucleic acid sequence that is involved with the viability of the potentially cross-reactive or interfering organism is the *murA* gene.

Particular examples of all methods disclosed herein are as follows:

In some examples, the compound is a PNA (peptide nucleic acid). In some examples, the compound is a peptide-PNA. In some examples, the peptide facilitates uptake of the oligonucleotide molecule into the microorganism. In some examples, the PNA targets a translation initiation region (TIR) of a gene. In some examples, the peptide-PNA targets a β-lactam resistance gene or a vancomycin resistance gene. In some examples, the peptide – PNA targets a bla_{KPC-3} gene, a bla_{NDM-1} gene, a bla_{SHV-18} gene, a *vanC* gene.

In some examples, contacting the sample with at least one compound comprising an oligonucleotide molecule comprises introducing into the microorganism a vector comprising the oligonucleotide molecule.

In some examples, the oligonucleotide molecule is a CRISPR RNA (crRNA). In some examples, the crRNA is expressed from a CRISPR/Cas system within the microorganism. In some examples, the crRNA targets a bla_{NDM-1} gene or a bla_{SHV-18} gene or transcript.

In some examples, the oligonucleotide molecule is an antisense oligonucleotide.

In some examples, the compound is a double-stranded RNA (dsRNA) comprising a sense strand and an antisense strand, wherein the antisense strand comprises the antisense molecule. In some examples, each strand of the dsRNA is 8 to 49 nucleotides in length (e.g., 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length), and optionally wherein each strand comprises a 3' T or TT. In some examples, at least one of the strands comprises at least one chemically modified nucleotide. In some examples, the chemically modified nucleotide is a 2'-modified nucleotide. In some examples, the 2'-modified nucleotide is a 2'-methyl substituted nucleotide or a 2'-amino substituted nucleotide.

In some examples, the specific nucleic acid sequence is a DNA sequence or an mRNA sequence.

In some examples, the presence of the detectable indication of viability indicates that the microorganism is viable. In some examples, the absence of the detectable indication of viability indicates that the microorganism is not viable.

In some examples, a method disclosed herein further comprises contacting the sample with a second compound comprising an oligonucleotide molecule targeting a second specific nucleic acid sequence involved with antimicrobial non-susceptibility to the antimicrobial agent in the microorganism.

In some examples, the microorganism is a prokaryote or a eukaryote.

In some examples, the detectable indication of viability is growth of the microorganism, a marker associated with the microorganism, or a detectable signal associated with the microorganism.

In some examples, a method disclosed herein further comprises contacting the sample with a reporter nucleic acid molecule encoding a reporter molecule, under conditions such that the reporter molecule enters the microorganism and provides the detectable indication of viability. In some examples, the reporter system is a liposome-based reporter system, a phage-based reporter system, or a non-replicative transduction particle-based reporter system.

In some examples, the at least one microorganism comprises a reporter nucleic acid molecule encoding a reporter molecule.

In some examples, a method disclosed herein further comprises contacting the sample with a non-replicative transduction particle (NRTP) comprising a reporter nucleic acid molecule encoding a reporter molecule, under conditions such that the NRTP inserts into the microorganism the reporter nucleic acid molecule and such that the reporter molecule provides the detectable indication of viability.

In some examples, the NRTP is produced from a bacterial cell packaging system that comprises a host bacteria cell, a first nucleic acid construct inside the host bacteria cell comprising a bacteriophage genome having a non-functional packaging initiation site sequence, wherein the non-functional packaging initiation site sequence prevents packaging of the bacteriophage genome into the NRTP, and a second nucleic acid construct inside the host bacteria cell and separate from the first nucleic acid construct comprising the reporter nucleic acid molecule having a reporter gene and a functional packaging initiation site sequence for facilitating packaging of a replicon of the reporter nucleic acid molecule into the NRTP, wherein the functional second packaging initiation site sequence on the second nucleic acid construct complements the non-functional packaging initiation site sequence in the bacteriophage genome on the first nucleic acid construct.

In some examples, the reporter nucleic acid molecule is a gene encoding a light-emitting molecule. In some examples, the gene is a luciferase gene.

In some examples, detecting the detectable indication of viability comprises detecting a presence or absence of the reporter molecule. In some examples, detecting the detectable indication of viability comprises detecting a presence or absence of a reaction mediated by the reporter molecule. In other examples, detecting the detectable indication of viability comprises detecting a conformation, activity, or other characteristic of the reporter molecule (e.g., fluorescence or ability to bind to or otherwise interact with another molecule).

In some examples, the microorganism is of the family Enterobacteriaceae, the genus Enterococcus, or the genus Candida.

In some examples, the microorganism is of the genus Escherichia, Mycobacterium, Staphylococcus, Listeria, Clostridium, Streptococcus, Helicobacter, Rickettsia, Haemophilus, Xenorhabdus, Acinetobacter, Bordetella, Pseudomonas, Aeromonas, Actinobacillus, Pasteurella, Vibrio,

Legionella, Bacillus, Calothrix, Methanococcus, Stenotrophomonas, Chlamydia, Neisseria, Salmonella, Shigella, Campylobacter or Yersinia.

In some examples, the antimicrobial is a β-lactam or vancomycin.

In some examples, the antimicrobial agent is of the group or class Penicillins, Cephalosporin, Carbapenems, Aminoglycosides, Fluoroquinolone, Lincosamide, Polymyxin, Tetracycline, Macrolide, Oxazolidinone, Streptogramins, Rifamycin, or Glycopeptide.

In some examples, the antimicrobial is Ampicillin, Ampicillin-sulbactam, Pipercillin-tazobactam, Oxacillin, Penicillin, Cefazolin, Cefepime, Cefotaxime, Ceftazidime, Ceftriaxone, Ceftaroline fosomil, Ertapenem, Imipenem, Meropenem, Amikacin, Gentamicin, Gentamicin Synergy, Streptomycin Synergy, Tobramycin, Ciprofloxacin, Levofloxacin, Clindamycin, Colistin, Daptomycin, Doxycycline, Erythromycin, Linezolid, Nitrofurantoin, Quinupristin-dalfopristin, Rifampin, Tigecycline, Trimethoprim-sulfamethoxazole, fosfomycin, cefoxitin, tetracycline, moxifloxacin, or tedizolid.

In certain examples, the microorganism is of the family Enterobacteriaceae and the antimicrobial agent is carbapenem.

In some examples, detecting the detectable indication of viability comprises observing the growth of the microorganism, optionally wherein growth is observed using cell culture.

In some examples, the compound further comprises a liposome.

In some examples, the sample is contacted with the antimicrobial agent prior to contacting the sample with the compound. In some examples, the sample is contacted with the compound prior to contacting the sample with the antimicrobial agent, or wherein the sample is contacted with the compound and the agent simultaneously.

In some examples, the sample, compound, and a reporter nucleic acid are contacted with each other in any sequential permutation or simultaneously.

Also disclosed herein is a kit for determining a mechanism for a non-susceptible phenotype for a microorganism that is not susceptible to an antimicrobial agent, comprising: an antimicrobial agent, wherein the antimicrobial agent can kill, inhibit the growth, or otherwise compromises the viability of one or more microorganisms; a compound comprising an oligonucleotide molecule targeting a specific nucleic acid sequence that is involved with a non-susceptible phenotype to the antimicrobial agent; and instructions for using the antimicrobial agent and the oligonucleotide molecule to determine the mechanism for the non-susceptible phenotype for the microorganism that is not susceptible to the antimicrobial agent based on the presence or absence of a detectable indication of viability associated with the microorganism when the microorganism is in contact with the antimicrobial agent and the oligonucleotide molecule, wherein the presence of the detectable indication of viability indicates that the specific nucleic acid sequence targeted by the oligonucleotide molecule is not related to the mechanism for the non-susceptible phenotype to the

antimicrobial agent, and wherein the absence of the detectable indication of viability indicates that the specific nucleic acid sequence targeted by the oligonucleotide molecule is related to the mechanism for the non-susceptible phenotype to the antimicrobial agent.

Also disclosed herein is a kit for determining the presence of an organism of interest, comprising: a compound comprising an oligonucleotide molecule targeting a specific nucleic acid sequence that is involved with the viability of the organism, wherein the specific nucleic acid sequence is unique to the organism; and instructions for using the oligonucleotide molecule to determine the presence of the organism based on the presence or absence of a detectable indication of viability associated with the organism when the organism is in contact with the oligonucleotide molecule, wherein the presence of the detectable indication of viability indicates that the organism of interest is not present in the sample, and wherein the absence of the detectable indication of viability indicates that the organism of interest may be present in the sample.

Also disclosed herein is an isolated microorganism that is not susceptible to at least one antimicrobial agent comprising: the antimicrobial agent, wherein the antimicrobial agent can kill, inhibit the growth, or otherwise compromise the viability of one or more microorganisms; at least one compound comprising an oligonucleotide molecule targeting at least one specific nucleic acid sequence that is involved with a non-susceptible phenotype to the antimicrobial agent; and a reporter, optionally wherein the reporter is a marker, a detectable signal, a reporter nucleic acid molecule encoding a reporter molecule, or a non-replicative transduction particle (NRTP) comprising a reporter nucleic acid molecule encoding a reporter molecule.

Also disclosed herein is a method of producing a microorganism disclosed herein, comprising: contacting the microorganism with an antimicrobial agent; contacting the microorganism with a compound comprising an oligonucleotide molecule; and contacting the microorganism with a reporter.

Also disclosed herein is an *in vitro* cell culture comprising a microorganism that is not susceptible to at least one antimicrobial agent, and further comprising: the antimicrobial agent, wherein the antimicrobial agent can kill, inhibit the growth, or otherwise compromise the viability of one or more microorganisms; at least one compound comprising an oligonucleotide molecule targeting at least one specific nucleic acid sequence that is involved with a non-susceptible phenotype to the antimicrobial agent; and a reporter, optionally wherein the reporter is a marker, a detectable signal, a reporter nucleic acid molecule encoding a reporter molecule, or a non-replicative transduction particle (NRTP) comprising a reporter nucleic acid molecule encoding a reporter molecule.

Also disclosed herein is a method of producing a cell culture, comprising contacting the culture with an antimicrobial agent; contacting the culture with a compound comprising an oligonucleotide molecule; and contacting the culture with a reporter.

Also disclosed herein is an isolated organism comprising: at least one compound comprising an oligonucleotide molecule targeting at least one specific nucleic acid sequence that is involved with the viability of the organism, wherein the specific nucleic acid sequence is unique to the organism; and a reporter, optionally wherein the reporter is a marker, a detectable signal, a reporter nucleic acid molecule encoding a reporter molecule, or a non-replicative transduction particle (NRTP) comprising a reporter nucleic acid molecule encoding a reporter molecule.

Also disclosed herein is a method of producing an isolated organism, comprising: contacting the organism with a compound comprising an oligonucleotide molecule; and contacting the organism with a reporter.

Also disclosed herein is an *in vitro* cell culture comprising an organism and further comprising: at least one compound comprising an oligonucleotide molecule targeting at least one specific nucleic acid sequence that is involved with the viability of the organism, wherein the specific nucleic acid sequence is unique to the organism; and a reporter, optionally wherein the reporter is a marker, a detectable signal, a reporter nucleic acid molecule encoding a reporter molecule, or a non-replicative transduction particle (NRTP) comprising a reporter nucleic acid molecule encoding a reporter molecule.

Also disclosed herein is a method of producing an *in vitro* cell culture, comprising: contacting the culture with a compound comprising an oligonucleotide molecule; and contacting the culture with a reporter.

Particular examples of all kits and all isolated microorganisms disclosed herein are as follows:

In some examples, the compound is a PNA (peptide nucleic acid). In some examples, the compound is a peptide-PNA. In some examples, the peptide facilitates uptake of the oligonucleotide molecule into the microorganism. In some examples, the PNA targets a translation initiation region (TIR) of a gene. In some examples, the peptide-PNA targets a β-lactam resistance gene or a vancomycin resistance gene. In some examples, the peptide – PNA targets a bla_{KPC-3} gene, a bla_{NDM-1} gene, a bla_{SHV-18} gene, a *vanC* gene.

In some examples, the oligonucleotide molecule is a CRISPR RNA (crRNA). In some aspects, the crRNA is expressed from a CRISPR/Cas system within the microorganism. In some examples, the crRNA targets a bla_{NDM-1} gene or a bla_{SHV-18} gene or transcript.

In some examples, the oligonucleotide molecule is an antisense oligonucleotide.

In some examples, the compound is a double-stranded RNA (dsRNA) comprising a sense strand and an antisense strand, wherein the antisense strand comprises the antisense molecule. In some examples, each strand of the dsRNA is 8 to 49 nucleotides in length (e.g., 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 nucleotides in length), and optionally wherein each strand comprises a 3' T or TT. In some examples, at least one of the strands comprises at least one chemically modified nucleotide. In some examples, the chemically modified nucleotide

is a 2'-modified nucleotide. In some examples, the 2'-modified nucleotide is a 2'-methyl substituted nucleotide or a 2'-amino substituted nucleotide.

In some examples, the specific nucleic acid sequence is a DNA sequence or an mRNA sequence.

In some examples, the presence of the detectable indication of viability indicates that the microorganism is viable. In some examples, the absence of the detectable indication of viability indicates that the microorganism is not viable.

In some examples, the microorganism is a prokaryote or a eukaryote.

In some examples, the detectable indication of viability is growth of the microorganism, a marker associated with the microorganism, or a detectable signal associated with the microorganism.

In some examples, the at least one microorganism comprises a reporter nucleic acid molecule encoding a reporter molecule.

In some examples, the kits further comprise a non-replicative transduction particle (NRTP) comprising a reporter nucleic acid molecule encoding a reporter molecule.

In some examples, the NRTP is produced from a bacterial cell packaging system that comprises a host bacteria cell, a first nucleic acid construct inside the host bacteria cell comprising a bacteriophage genome having a non-functional packaging initiation site sequence, wherein the non-functional packaging initiation site sequence prevents packaging of the bacteriophage genome into the NRTP, and a second nucleic acid construct inside the host bacteria cell and separate from the first nucleic acid construct comprising the reporter nucleic acid molecule having a reporter gene and a functional packaging initiation site sequence for facilitating packaging of a replicon of the reporter nucleic acid molecule into the NRTP, wherein the functional second packaging initiation site sequence on the second nucleic acid construct complements the non-functional packaging initiation site sequence in the bacteriophage genome on the first nucleic acid construct.

In some examples, the reporter nucleic acid molecule is a gene encoding a light-emitting molecule. In some examples, the gene is a luciferase gene.

In some examples, detecting the detectable indication of viability comprises detecting a presence or absence of the reporter molecule. In some examples, detecting the detectable indication of viability comprises detecting a presence or absence of a reaction mediated by the reporter molecule. In other examples, detecting the detectable indication of viability comprises detecting a conformation, activity, or other characteristic of the reporter molecule (e.g., fluorescence or ability to bind to or otherwise interact with another molecule).

In some examples, the microorganism is of the family Enterobacteriaceae, the genus Enterococcus, or the genus Candida.

In some examples, the microorganism is of the genus Escherichia, Mycobacterium, Staphylococcus, Listeria, Clostridium, Streptococcus, Helicobacter, Rickettsia, Haemophilus, Xenorhabdus, Acinetobacter, Bordetella, Pseudomonas, Aeromonas, Actinobacillus, Pasteurella, Vibrio,

Legionella, Bacillus, Calothrix, Methanococcus, Stenotrophomonas, Chlamydia, Neisseria, Salmonella, Shigella, Campylobacter or Yersinia.

In some examples, the antimicrobial is a β-lactam or vancomycin.

In some examples, the antimicrobial agent is of the group or class Penicillins, Cephalosporin, Carbapenems, Aminoglycosides, Fluoroquinolone, Lincosamide, Polymyxin, Tetracycline, Macrolide, Oxazolidinone, Streptogramins, Rifamycin, or Glycopeptide.

In some examples, the antimicrobial is Ampicillin, Ampicillin-sulbactam, Pipercillin-tazobactam, Oxacillin, Penicillin, Cefazolin, Cefepime, Cefotaxime, Ceftazidime, Ceftriaxone, Ceftaroline fosomil, Ertapenem, Imipenem, Meropenem, Amikacin, Gentamicin, Gentamicin Synergy, Streptomycin Synergy, Tobramycin, Ciprofloxacin, Levofloxacin, Clindamycin, Colistin, Daptomycin, Doxycycline, Erythromycin, Linezolid, Nitrofurantoin, Quinupristin-dalfopristin, Rifampin, Tigecycline, Trimethoprim-sulfamethoxazole, fosfomycin, cefoxitin, tetracycline, moxifloxacin, or tedizolid.

In certain examples, the microorganism is of the family Enterobacteriaceae and the antimicrobial agent is carbapenem.

In some examples, detecting the detectable indication of viability comprises observing the growth of the microorganism, optionally wherein growth is observed using cell culture.

In some examples, the compound further comprises a liposome.

Also disclosed herein is a peptide nucleic acid (PNA) molecule for inhibiting the expression of a gene involved with carbapenem non-susceptibility in Enterobacteriaceae, the PNA molecule comprising the nucleotide sequence of SEQ ID NO: 2 or SEQ ID NO: 3.

Also disclosed herein is a PNA molecule for inhibiting the expression of a gene involved with vancomycin non-susceptibility in Enterococcus, the PNA molecule comprising the nucleotide sequence of SEQ ID NO: 6.

Also disclosed herein is an antisense molecule for inhibiting the expression of an internal transcribed spacer region in Candida, the antisense molecule comprising the nucleotide sequence of SEQ ID NO: 7, SEQ ID NO: 8, or SEQ ID NO: 9.

BRIEF DESCRIPTION OF THE DRAWINGS

These and other features, aspects, and advantages of the present disclosure will become better understood with regard to the following description, and accompanying drawings, where:

Fig. 1 shows the effect of *E. coli*-specific peptide-PNA on the growth of *E. coli.*

Fig. 2 shows the effect of *E. coli*-specific peptide-PNA on the growth of *K. pneumoniae.*

Fig. 3 shows the effect of *E. coli*-specific peptide-PNA on the luminescence signal from *E. coli* and *K. pneumoniae* using an Enterobacteriaceae luminescence reporter.

Fig. 4 depicts an example of typical meropenem/peptide-PNA disk diffusion results for *E. coli* 1289011, 1289012, 1289014, and 1289018.

Fig. 5 depicts an example of results of a peptide PNA cell-reporter AST assay where + indicates that the reporter assay produced a positive result and - indicates that the reporter assay produced a negative result.

Fig. 6 depicts an example of results of a CRISPR Cas9 cell-reporter AST assay where + indicates that the reporter assay produced a positive result and - indicates that the reporter assay produced a negative result.

Fig. 7 depicts an example of results of a VRE cell-reporter assay where + indicates that the reporter assay produced a positive result and - indicates that the reporter assay produced a negative result.

Fig. 8 includes a list of ITS2 sequences that can be used for producing 2'-OMe modified antisense oligonucleotides (AON) targeting each species.

Fig. 9 depicts an assay setup for the combination of AON used for *Candida spp.* identification.

DETAILED DESCRIPTION OF THE INVENTION

Terms used in the claims and specification are defined as set forth below unless otherwise specified.

As used herein, "reporter nucleic acid molecule" refers to a nucleotide sequence comprising a DNA or RNA molecule. The reporter nucleic acid molecule can be naturally occurring or an artificial or synthetic molecule. In some embodiments, the reporter nucleic acid molecule

CLAIMS

1. A method for determining a mechanism for an antimicrobial susceptibility phenotype, comprising:
   - providing a sample comprising at least one microorganism that is not susceptible to at least one antimicrobial agent;
   - contacting the sample with the antimicrobial agent, wherein the antimicrobial agent can kill, inhibit the growth, or otherwise compromise the viability of one or more microorganisms;
   - contacting the at least one microorganism with at least one compound comprising an oligonucleotide molecule targeting at least one specific nucleic acid sequence that is involved in a non-susceptible phenotype to the antimicrobial agent;
   - contacting the sample with a non-replicative transduction particle (NRTP) comprising a reporter nucleic acid molecule encoding a reporter molecule under conditions such that the NRTP inserts the reporter nucleic acid molecule encoding the reporter molecule into the microorganism and such that the reporter molecule provides the detectable indication of viability; and
   - determining the mechanism for the antimicrobial susceptibility phenotype of the microorganism based on the presence or absence of a detectable indication of viability associated with the microorganism when the microorganism is in contact with the antimicrobial agent and the oligonucleotide molecule, wherein the presence of the detectable indication of viability indicates that the specific nucleic acid sequence targeted by the oligonucleotide molecule is not related to the mechanism for the antimicrobial susceptibility phenotype to the antimicrobial agent, and wherein the absence of the detectable indication of viability indicates that the specific nucleic acid sequence targeted by the oligonucleotide molecule is related to the mechanism for the antimicrobial susceptibility phenotype to the antimicrobial agent.

2. The method of claim 1, wherein the reporter nucleic acid molecule is a gene encoding a light-emitting molecule.

3. The method of any one of claims 1 to 2, wherein the NRTP is produced from a bacterial cell packaging system that comprises a host bacteria cell, a first nucleic acid construct inside the host bacteria cell comprising a bacteriophage genome having a non-functional packaging initiation site sequence, wherein the non-functional

packaging initiation site sequence prevents packaging of the bacteriophage genome into the NRTP, and a second nucleic acid construct inside the host bacteria cell and separate from the first nucleic acid construct comprising the reporter nucleic acid molecule having a reporter gene and a functional packaging initiation site sequence for facilitating packaging of a replicon of the reporter nucleic acid molecule into the NRTP, wherein the functional second packaging initiation site sequence on the second nucleic acid construct complements the non-functional packaging initiation site sequence in the bacteriophage genome on the first nucleic acid construct.

4. The method of any one of claims 1 to 3, wherein the compound is selected from the group consisting of a peptide nucleic acid (PNA), a peptide-PNA, a CRISPR RNA (crRNA), an antisense oligonucleotide, and a double-stranded RNA (dsRNA).

5. The method of any one of claims 1 to 4, wherein the at least one specific nucleic acid sequence that is involved in a non-susceptible phenotype to the antimicrobial agent is selected from the group consisting of a bla_{KPC-3} gene, a bla_{NDM-1} gene, a bla_{SHV-18} gene, and a *vanC* gene.

6. The method of any one of claims 1 to 5, wherein the microorganism is of the family Enterobacteriaceae, the genus *Enterococcus*, or the genus *Candida*.

7. The method of any one of claims 1 to 6, wherein the antimicrobial is a β-lactam or vancomycin.

8. A method for determining a mechanism involved in a non-susceptible phenotype, comprising:

- providing a sample comprising an Enterobacteriaceae that is not susceptible to carbapenem;
- contacting the sample with carbapenem;
- contacting the Enterobacteriaceae with an oligonucleotide molecule that targets a gene associated with carbapenem resistance;
- contacting the sample with a non-replicative transduction particle (NRTP) comprising a reporter nucleic acid molecule encoding a reporter molecule under conditions such that the NRTP inserts the reporter nucleic acid molecule encoding the reporter molecule into the Enterobacteriaceae and such that the reporter molecule provides the detectable indication of viability; and
- determining the mechanism of carbapenem non-susceptibility for the Enterobacteriaceae based on the presence or absence of a detectable indication of

viability associated with the Enterobacteriaceae when the Enterobacteriaceae is in contact with the carbapenem and the oligonucleotide molecule, wherein the presence of the detectable indication of viability indicates that the gene targeted by the oligonucleotide molecule is not related to the mechanism of carbapenem non-susceptibility, and wherein the absence of the detectable indication of viability indicates that the gene targeted by the oligonucleotide molecule is related to the mechanism of carbapenem non-susceptibility.

9. The method of claim 8, wherein the reporter nucleic acid molecule is a gene encoding a light-emitting molecule.

10. The method of any one of claims 8 to 9, wherein the NRTP is produced from a bacterial cell packaging system that comprises a host bacteria cell, a first nucleic acid construct inside the host bacteria cell comprising a bacteriophage genome having a non-functional packaging initiation site sequence, wherein the non-functional packaging initiation site sequence prevents packaging of the bacteriophage genome into the NRTP, and a second nucleic acid construct inside the host bacteria cell and separate from the first nucleic acid construct comprising the reporter nucleic acid molecule having a reporter gene and a functional packaging initiation site sequence for facilitating packaging of a replicon of the reporter nucleic acid molecule into the NRTP, wherein the functional second packaging initiation site sequence on the second nucleic acid construct complements the non-functional packaging initiation site sequence in the bacteriophage genome on the first nucleic acid construct.

11. The method of any one of claims 8 to 10, wherein the oligonucleotide molecule is selected from the group consisting of a peptide nucleic acid (PNA), a peptide-PNA, a CRISPR RNA (crRNA), an antisense oligonucleotide, and a double-stranded RNA (dsRNA).

12. The method of claim 11 wherein the oligonucleotide molecule is selected from the group consisting of SEQ ID NO 2 or SEQ ID NO 3, or wherein the oligonucleotide molecule is a CRISPR/Cas9 System comprising SEQ ID NO 4or SEQ ID NO 5.

13. A method for reducing the amount of potentially cross-reactive or interfering organisms in an assay designed to detect a detectable indication of viability of a target organism comprising:

- providing a sample comprising at least one organism that is potentially cross-reactive or interfering in said assay designed to detect the detectable indication of viability of the target organism;
- contacting the cross-reactive or interfering organism with at least one compound comprising an oligonucleotide molecule targeting at least one specific nucleic acid sequence that is involved with the viability of the potentially cross-reactive or interfering organism, wherein the specific nucleic acid sequence is unique to the potentially cross-reactive or interfering organism; and causing the potentially cross-reactive or interfering organism to lose viability; and
- contacting the sample with a non-replicative transduction particle (NRTP) comprising a reporter nucleic acid molecule encoding a reporter molecule under conditions such that the NRTP inserts the reporter nucleic acid molecule encoding the reporter molecule into the target organism and such that the reporter molecule provides a detectable indication of viability of the target organism.

14. The method of claim 13, wherein the reporter nucleic acid molecule is a gene encoding a light-emitting molecule.

15. The method of any one of claims 13 to 14, wherein the compound is selected from the group consisting of a peptide nucleic acid (PNA), a peptide-PNA, a CRISPR RNA (crRNA), an antisense oligonucleotide, and a double-stranded RNA (dsRNA).

16. The method of any one of claims 13 to 15, wherein the NRTP is produced from a bacterial cell packaging system that comprises a host bacteria cell, a first nucleic acid construct inside the host bacteria cell, comprising of a bacteriophage genome having a non-functional packaging initiation site sequence, wherein the non-functional packaging initiation site sequence prevents packaging of the bacteriophage genome into the NRTP, and a second nucleic acid construct inside the host bacteria cell and separate from the first nucleic acid construct, comprising of the reporter nucleic acid molecule having a reporter gene and a functional packaging initiation site sequence for facilitating packaging of a replicon of the reporter nucleic acid molecule into the NRTP, wherein the functional second packaging initiation site sequence on the second nucleic acid construct complements the non-functional packaging initiation site sequence in the bacteriophage genome on the first nucleic acid construct.

17. The method of any one of claims 13 to 16, wherein the target organism is of the family Enterobacteriaceae.

18. The method of any one of claims 13 to 17, wherein the at least one specific nucleic acid sequence that is involved with the viability of the potentially cross-reactive or interfering organism is the *murA* gene.