organism and thus requires prior isolation of the organism and cannot determine susceptibility if the mechanism responsible is not enzymatic.

Automated systems exist that provide identification and susceptibility information in an integrated system. The combination of peptide nucleic acid (PNA) fluorescence in situ hybridization (FISH) for organism identification and automated microscopy for the monitoring of the organism's growth rate in the presence of antibiotics is one example of such a system (Metzger, S., R.A. Frobel, and W.M. Dunne Jr, *Rapid simultaneous identification and quantitation of Staphylococcus aureus and Pseudomonas aeruginosa directly from bronchoalveolar lavage specimens using automated microscopy*, Diagnostic Microbiology and Infectious Disease, 2014. 79(2): p. 160-165). However, this system lacks the ability to determine the mechanisms involved in the observed susceptibility phenotype.

Finally, traditional culture techniques may be used to identify organisms and determine the mechanisms involved in imparting a susceptibility phenotype. An example of such an assay is the Modified Hodge Test used for determining the presence of carbapenemases (Lee, K., et al., *Modified Hodge and EDTA-disk synergy tests to screen metallo-β-lactamase-producing strains of Pseudomonas and Acinetobactet species*, Clinical Microbiology and Infection, 2001. **7**(2): p. 88-91). However, these techniques rely on observations derived from the antibiotic susceptibility phenotype of an organism and cannot detect the presence of a mechanism involved in the phenotype if the phenotype is not expressed.

The limitations of existing technologies thus requires information from multiple assays and systems in order to provide identification, phenotypic susceptibility determination, and determination of the mechanisms involved in imparting the susceptibility phenotype. Because of this, healthcare providers must purchase and operate different systems in order to acquire this information – a costly and complicated endeavor. Due to these limitations, there is a need for a single assay that provides identification, phenotypic susceptibility determination, and determination of the mechanisms involved in imparting the susceptibility phenotype.

SUMMARY

Disclosed herein are methods and compositions for determining the presence or absence of a mechanism of antimicrobial resistance in a sample. In one aspect, the present invention refers to In one example, there is disclosed a method for determining the presence or absence of a mechanism of antimicrobial resistance in a microorganism that is not susceptible to an antimicrobial in a sample, comprising providing a sample comprising a

microorganism of interest; contacting at least a portion of said sample with an inhibitor, wherein said inhibitor inhibits a mechanism of conferring non-susceptibility in said microorganism; contacting said portion of said sample comprising said inhibitor with an antimicrobial agent, wherein said antimicrobial agent is a compound that kills, inhibits the growth, or otherwise compromises the viability of one or more microorganisms; contacting said portion of said sample with a live cell reporter capable of generating a detectable signal; and detecting the presence or absence of a signal from said portion of said sample, wherein the presence of said signal indicates that the mechanism of antimicrobial resistance inhibited by said inhibitor is not present in said microorganism, and wherein the absence of said signal indicates that the mechanism of antimicrobial resistance inhibited by said inhibitor is present in said microorganism.

In another aspect, the present invention refers to a method for determining the presence or absence of a mechanism of antimicrobial resistance in a microorganism in a sample, comprising, providing a sample comprising a microorganism of interest; contacting a first portion of said sample with an antimicrobial agent, wherein said antimicrobial agent is a compound that kills, inhibits the growth, or otherwise compromises the viability of one or more microorganisms; contacting said first portion of said sample with a live cell reporter capable of generating a detectable signal; detecting the presence or absence of a signal from said first portion of said sample comprising said antimicrobial agent and said live cell reporter, wherein the absence of said signal indicates that said microorganism is susceptible to said antimicrobial agent, and wherein the presence of said signal indicates that said microorganism is non-susceptible to said antimicrobial agent; contacting a second portion of said sample with an inhibitor, wherein said inhibitor inhibits a mechanism of conferring non-susceptibility to said antimicrobial agent in said microorganism; contacting said second portion of said sample comprising said inhibitor with said antimicrobial agent; contacting said second portion of said sample with a live cell reporter capable of generating a detectable signal; and detecting the presence or absence of a signal from said second portion of said sample, wherein the presence of said signal indicates that the mechanism of antimicrobial resistance inhibited by said inhibitor is not present in said microorganism, and wherein the absence of said signal indicates that the mechanism of antimicrobial resistance inhibited by said inhibitor is present in said microorganism.

In still another aspect, the present invention refers to a method for determining the presence or absence of a mechanism of antimicrobial resistance in a microorganism in a sample,

comprising, providing a sample comprising a microorganism of interest; contacting a first portion of said sample with an antimicrobial agent and a live cell reporter capable of generating a detectable signal when in the presence of a substrate, wherein said antimicrobial agent is a compound that kills, inhibits the growth of, or otherwise compromises the viability of one or more microorganisms; contacting a second portion of said sample with a caged substrate and a live cell reporter capable of generating a detectable signal, wherein the caged substrate is un-caged by a mechanism associated with a non-susceptibility phenotype to said antimicrobial agent; and detecting the presence or absence of a signal from said first portion and from said second portion, wherein the absence of said signal from said first portion indicates that the microorganism is susceptible to said antimicrobial agent, wherein the presence of said signal from said first portion indicates that the microorganism is non-susceptible to said antimicrobial agent, wherein the presence of said signal from said second portion indicates the presence of said mechanism associated with said non-susceptibility phenotype to said antimicrobial agent, and wherein the absence of said signal from said second portion indicates the absence of said mechanism associated with said non-susceptibility phenotype to said antimicrobial agent.

In another example, there is disclosed a kit for determining a mechanism of antimicrobial resistance for a microorganism to an antimicrobial agent comprising an antimicrobial agent, wherein the antimicrobial agent is a compound that kills, inhibits the growth of, or otherwise compromises the viability of the growth of one or more microorganisms; a non-replicative transduction particle (NRTP) comprising a reporter nucleic acid molecule encoding a reporter molecule; a caged substrate capable of entering the microorganism and becoming an un-caged substrate in the presence of an enzyme related to a mechanism of resistance to the antimicrobial agent, wherein the un-caged substrate reacts with the reporter molecule to produce a detectable signal, wherein detection of the detectable signal confirms a presence of the microorganism in the sample; and instructions for using the antimicrobial agent, the NRTP, and the caged substrate to determine the mechanism of antimicrobial resistance for the microorganism 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, the NRTP and the caged substrate, wherein the presence of a detectable indication of viability indicates that the microorganism is viable and that the enzyme related to the mechanism of resistance to the antimicrobial agent is expressed by the microorganism, and wherein the absence of an indication of

viability indicates that the microorganism is not viable and that the enzyme related to the mechanism of resistance to the antimicrobial agent is not expressed by the microorganism.

In another example, there is disclosed an *in vitro* cell culture, a plurality of cells in the cell culture comprising a non-replicative transduction particle (NRTP) comprising a reporter nucleic acid molecule encoding a reporter molecule; and a caged substrate capable of becoming uncaged in the presence of enzymatic activity associated with a mechanism of antimicrobial resistance, wherein said uncaged substrate is capable of reacting with said reporter molecule to produce a detectable signal.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

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

Figure 1 illustrates a reporter system for the detection of intracellular enzymes within viable cells that employs caged substrate molecules that can be un-caged by a target intracellular enzyme, according to an embodiment.

Figure 2 shows % RLU signal from NRTP detection of NDM-1-expressing *E. coli* in the presence of various additives, normalized to signal from No Antibiotic.

Figure 3 shows % RLU signal from NRTP detection of KPC-expressing *E. coli* in the presence of various additives, normalized to signal from No Antibiotic

Figure 4 illustrates a bacterial luciferase-mediated luminescence reaction, according to an embodiment.

Figure 5 illustrates the synthesis of a meropenem-caged decanal, according to an embodiment.

Figure 6 illustrates an alternative mechanism of synthesis of a meropenem-caged decanal, according to another embodiment.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

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