

SUMMARY OF THE INVENTION

[0005] The invention is based upon an ultra-sensitive method for detecting multi-drug resistant bacterial colonization or infection in a subject. In addition, the invention relates to methods for the rapid detection and characterization of MDROs, bacteria, viruses, fungi, yeasts, and molecular, phenotypic, and host biomarkers associated with infectious diseases, through high density matrix gene testing in order to provide the most comprehensive, high resolution profile of colonization, infection, and resistance pattern in a test sample. In various examples the method includes isolating a nucleic acid sample from a biological sample obtained from the patient by amplifying the antibiotic resistant genes KPC, NDM, OXA, VIM, IMP, CTX-M and VanA by contacting the nucleic acid sample with one or more amplification primers that specifically hybridize with the each of the antibiotic resistant genes to provide an enriched nucleic acid sample; detecting the presence of the antibiotic gene by contacting the nucleic acid sample with one or more detection primers that specifically hybridize with the each of the antibiotic resistant genes to in the enriched nucleic acid sample; classifying the patient as having a multi-drug resistant bacterial colonization or infection when one or more of the antibiotic resistant genes are identified in the enriched nucleic acid sample; and providing a contact precautions recommendation for the patient having a multi-drug resistant bacterial colonization or infection. Optionally, the antibiotic resistant genes further include one or more of IMI, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC, CMY, MIR, ACT, DHA, MOX, FOX, or CFE.

[0005a] Disclosed is a method of screening a patient for multi-drug resistant bacterial colonization or infection comprising: isolating a nucleic acid sample from a biological sample obtained from the patient; amplifying at least one antibiotic gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting the nucleic acid sample with one or more amplification primers that specifically hybridizes to a substantially homologous region common to at least two antibiotic gene subtypes in each antibiotic resistant gene family to provide an enriched nucleic acid sample; detecting the presence of the at least one antibiotic gene subtype by contacting the enriched nucleic acid sample with one or more detection primer that specifically hybridizes to the substantially homologous region or to another substantially homologous region common to at

least two antibiotic gene subtypes in each antibiotic resistant gene family; classifying the patient as having a multi-drug resistant bacterial colonization or infection when at least one antibiotic gene subtype from each of the antibiotic resistant gene families is identified in the enriched nucleic acid sample; and providing a contact precautions recommendation for the patient having a multi-drug resistant bacterial colonization or infection.

[0005b] In one aspect, the present invention provides a method of screening a patient for multi-drug resistant bacterial colonization or infection, the method comprising: isolating a nucleic acid sample from a biological sample obtained from the patient; amplifying at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting the nucleic acid sample with a plurality of sets of amplification primers, wherein each set of amplification primers specifically hybridizes to a substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families to provide an enriched nucleic acid sample, wherein the nucleic acid sample is contacted by an internal amplification control nucleic acid prior to amplification; detecting the presence of the at least one antibiotic resistant gene subtype by contacting the enriched nucleic acid sample with a plurality of sets of detection primers, wherein each set of detection primers specifically hybridizes to the substantially identical region or to another substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families, wherein said contacting results in amplification of the substantially identical region or amplification of another substantially identical region common to at least two antibiotic resistant gene subtypes within each antibiotic resistant gene family; classifying the patient as having a multi-drug resistant bacterial colonization or infection when at least one antibiotic resistant gene subtype from each of the antibiotic resistant gene families is identified in the enriched nucleic acid sample; and providing a contact precautions recommendation for the patient having a multi-drug resistant bacterial colonization or infection.

[0006] Optionally, the method further includes making a treatment recommendation for the patient. Treatment recommendation include recommending that the patient does not receive a carbapenem antibiotic when KPC, NDM, OXA, VIM, IMP, SME, SPC, IMI, NMC, or CcrA is detected; recommending that the patient does not receive a cephalosporin antibiotic when CTM-X, PER, VEB, GES, BES, SFO, TLA, TEM with amino acid substitutions E104K, R164H,

R164S, R164C, G238S or E240K, or SHV with amino acid substitutions G156D, G238S or E240K, is detected recommending that the patient does not receive a beta-lactamase inhibitor-beta-lactam combination when ACC, MOX, CMY, CFE, ACT, DHA or FOX is detected; or recommending that the patient does not receive vancomycin when VanA is detected.

[0007] In some examples the method further includes testing the biological sample to identify the phenotype of the multi-drug resistant organism and/or culturing the biological sample and confirming drug resistance.

[0008] In other examples the disclosure includes methods of making a treatment recommendation for a subject known to or suspected of being colonized with or having a bacterial infection isolating a nucleic acid sample from a biological sample obtained from the patient by amplifying the antibiotic resistant genes KPC, NDM, OXA, VIM, IMP, CTX-M and VanA and optionally one or more of IMI, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC, CMY, MIR, ACT, DHA, MOX, FOX, or CFE by contacting the nucleic acid sample with one or more amplification primers that specifically hybridize with the each of the antibiotic resistant genes to provide an enriched nucleic acid sample; detecting the presence of the antibiotic gene by contacting the nucleic acid sample with one or more detection primers that specifically hybridize with the each of the antibiotic resistant genes to in the enriched nucleic acid sample recommending that the patient does not receive a carbapenem antibiotic when KPC, NDM, OXA, VIM, SFC, IMP, SME, IMI, NMC, or CcrA is detected; recommending that the patient does not receive a cephalosporin antibiotic when CTM-X, PER, VEB, GES, BES, SFO, TLA, TEM with amino acid substitutions E104K, R164H, R164S, R164C, G238S or E240K, or SHV with amino acid substitutions G156D, G238S or E240K, is detected recommending that the patient does not receive a beta-lactamase inhibitor-beta-lactam combination when ACC, MOX, CMY, CFE, ACT, DHA or FOX is detected; or recommending that the patient does not receive vancomycin when VanA is detected.

[0008a] Also disclosed is a method of making a treatment recommendation for a subject known to or suspected of being colonized with or having a bacterial infection comprising: isolating a nucleic acid sample from a biological sample obtained from the patient; amplifying at least one antibiotic gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting the nucleic acid sample with one or more amplification primers that specifically hybridizes to a substantially homologous region common to at least two antibiotic gene subtypes in each antibiotic resistant gene family to provide an enriched nucleic acid sample; detecting the presence of the at least one antibiotic gene subtype by contacting the enriched nucleic acid sample with one or more detection primer that specifically hybridizes to the substantially homologous region or to another

substantially homologous region common to at least two antibiotic resistant gene subtypes in each antibiotic resistant gene family; recommending that the patient does not receive a carbapenem antibiotic when at least one antibiotic gene subtype from each of the KPC, NDM, OXA, VIM, and IMP antibiotic resistant gene families is detected; recommending that the patient does not receive a cephalosporin antibiotic when at least one antibiotic gene subtype from the CTM-X antibiotic resistant gene family is detected; or recommending that the patient does not receive vancomycin when at least one antibiotic gene subtype from the VanA antibiotic resistant gene family is detected.

[0008b] In another aspect, the present invention provides a method of making a treatment recommendation for a subject known to be or suspected of being colonized with or having a bacterial infection comprising: isolating a nucleic acid sample from a biological sample obtained from the patient; amplifying at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting the nucleic acid sample with a plurality of sets of amplification primers, wherein each set of amplification primers specifically hybridizes to a substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families to provide an enriched nucleic acid sample, wherein the nucleic acid sample is contacted by an internal amplification control nucleic acid prior to amplification; detecting the presence of the at least one antibiotic resistant gene subtype by contacting the enriched nucleic acid sample with a plurality of sets of detection primers, wherein each set of detection primers specifically hybridizes to the substantially identical region or to another substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families, wherein said contacting results in amplification of the substantially identical region or amplification of another substantially identical region common to at least two antibiotic resistant gene subtypes within each antibiotic resistant gene family; and (i) recommending that the patient does not receive a carbapenem antibiotic when at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, and IMP antibiotic resistant gene families is detected; (ii) recommending that the patient does not receive a cephalosporin antibiotic when at least one antibiotic resistant gene subtype from the CTX-M antibiotic resistant gene family is detected; or (iii) recommending that the patient does not receive vancomycin when at least one antibiotic resistant gene subtype from the VanA antibiotic resistant gene family is detected.

[0009] Also disclosed are methods of making a treatment recommendation for a healthy subject by isolating a nucleic acid sample from a biological sample obtained from the patient; amplifying the antibiotic resistant genes KPC, NDM, OXA, VIM, IMP, CTX-M and VanA and optionally one or more of IMI, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC, CMY, MIR, ACT, DHA, MOX, FOX, or CFE by contacting the nucleic acid sample with one or more amplification primers that specifically hybridize with the each of the antibiotic resistant genes to provide an enriched nucleic acid sample; detecting the presence of the antibiotic gene by contacting the nucleic acid sample with one or more detection primers that specifically hybridize with the each of the antibiotic resistant genes to in the enriched nucleic acid sample and recommending that the

patient does not receive a carbapenem antibiotic when KPC, NDM, OXA, VIM, SFC, IMP, SME, IMI, NMC, or CcrA is detected; recommending that the patient does not receive a cephalosporin antibiotic when CTM-X, PER, VEB, GES, BES, SFO, TLA, TEM with amino acid substitutions E104K, R164H, R164S, R164C, G238S or E240K, or SHV with amino acid substitutions G156D, G238S or E240K, is detected recommending that the patient does not receive a beta-lactamase inhibitor-beta-lactam combination when ACC, MOX, CMY, CFE, ACT, DHA or FOX is detected; or recommending that the patient does not receive vancomycin when VanA is detected.

[00010] The disclosure further includes methods of identifying the emergence of a multi-drug resistant organism in a population of subjects identifying one or more antibiotic resistant genes selected from KPC, NDM, OXA, VIM, IMP, CTX-M and VanA and optionally one or more of IMI, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC, CMY, MIR, ACT, DHA, MOX, FOX, or CFE in a plurality of subject samples wherein identification of an antibiotic resistant genes in the plurality of subject samples indicates the emergence of a multi-drug resistant organism in a population.

[00010a] Also disclosed is a method of making a treatment recommendation for a healthy subject comprising: isolating a nucleic acid sample from a biological sample obtained from the subject; amplifying at least one antibiotic gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting the nucleic acid sample with one or more amplification primers that specifically hybridizes to a substantially homologous region common to at least two antibiotic gene subtypes in each antibiotic resistant gene family to provide an enriched nucleic acid sample; detecting the presence of the at least one antibiotic gene subtype by contacting the enriched nucleic acid sample with one or more detection primer that specifically hybridizes to the substantially homologous region or to another substantially homologous region common to at least two antibiotic gene subtypes in each antibiotic resistant gene family; recommending that the subject does not receive a carbapenem antibiotic when at least one antibiotic gene subtype from each of the KPC, NDM, OXA, VIM, and IMP antibiotic resistant gene families is detected; recommending that the subject does not receive a beta-lactam antibiotic when at least one antibiotic gene subtype from the CTM-X antibiotic resistant gene family is detected; or

recommending that the subject does not receive vancomycin when at least one antibiotic gene subtype from the VanA antibiotic resistant gene family is detected.

[00010b] Also disclosed is a method of identifying the emergence of a multi-drug resistant organism in a population of subjects comprising identifying one or more antibiotic gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families in a plurality of subject samples wherein identification of the one or more an antibiotic gene subtype in the plurality of subject samples indicates the emergence of a multi-drug resistant organism in the population of subjects.

[00010c] In yet another aspect, the present invention provides a method of making a treatment recommendation for an asymptomatic subject comprising: isolating a nucleic acid sample from a biological sample obtained from the subject; amplifying at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting the nucleic acid sample with a plurality of sets of amplification primers, wherein each set of amplification primers specifically hybridizes to a substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families to provide an enriched nucleic acid sample, wherein the nucleic acid sample is contacted by an internal amplification control nucleic acid prior to amplification; detecting the presence of the at least one antibiotic resistant gene subtype by contacting the enriched nucleic acid sample with a plurality of sets of detection primers, wherein each set of detection primers specifically hybridizes to the substantially identical region or to another substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families, wherein said contacting results in amplification of the substantially identical region or amplification of another substantially identical region common to at least two antibiotic resistant gene subtypes within each antibiotic resistant gene family; and (i) recommending that the subject does not receive a carbapenem antibiotic when at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, and IMP antibiotic resistant gene families is detected; (ii) recommending that the subject does not receive a beta-lactam antibiotic when at least one antibiotic resistant gene subtype from the CTX-M antibiotic resistant gene family is detected; or (iii) recommending that the subject does not receive vancomycin when at least one antibiotic resistant gene subtype from the VanA antibiotic resistant gene family is detected.

[00010d] In yet another aspect, the present invention provides a method of identifying the emergence of a multi-drug resistant organism in a population of subjects, the method comprising: isolating a plurality of nucleic acid samples from a plurality of biological samples obtained from a plurality of subjects in the population of subjects; amplifying at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting each nucleic acid sample with a plurality of sets of amplification primers, wherein each set of amplification primers specifically hybridizes to a substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families to provide a plurality of enriched nucleic acid samples, wherein each nucleic acid sample is contacted by an internal amplification control nucleic acid prior to amplification; and identifying one or more antibiotic resistant gene subtypes from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families in the plurality of the enriched nucleic acid samples, wherein identification of the one or more antibiotic resistant gene subtypes in the plurality of the enriched nucleic acid samples indicates the emergence of a multi-drug resistant organism in the population of subjects.

[00011] Also provided are methods of screening a patient for multi-drug resistant bacterial colonization or infection by isolating a nucleic acid sample from a biological sample obtained from the patient such that the sample is substantially free of protein, cellular debris and or PCR inhibitors and identifying one or more antibiotic resistant genes selected from KPC, NDM, OXA, VIM, IMP, CTX-M and VanA and optionally one or more of IMI, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC, CMY, MIR, ACT, DHA, MOX, FOX, or CFE in the sample wherein identification of an antibiotic resistant genes indicates the subject is colonized or infected with a multi-drug resistant bacterial organism.

[00011a] Also disclosed is a method of screening a patient for a multi-drug resistant bacterial colonization or infection comprising: isolating a nucleic acid sample from a biological sample obtained from the patient such that the sample is substantially free of protein, cellular debris, and /or PCR inhibitors; and identifying one or more antibiotic gene subtype selected from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistance gene families in said sample, wherein identification of the one or more antibiotic gene subtype indicates that the patient has been colonized or infected with a multi-drug resistant bacterial organism.

[00011b] In yet another aspect, the present invention provides a method of screening a patient for a multi-drug resistant bacterial colonization or infection, the method comprising: isolating a nucleic acid sample from a biological sample obtained from the patient such that the nucleic acid sample is substantially free of protein, cellular debris, and /or PCR inhibitors; amplifying at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting the nucleic acid sample with a plurality of sets of amplification primers, wherein each set of amplification primers specifically hybridizes to a substantially identical region common to at least two antibiotic gene subtypes within one of the antibiotic resistant gene families to provide an enriched nucleic acid sample, wherein the nucleic acid sample is contacted by an internal amplification control nucleic acid prior to amplification; and identifying one or more antibiotic resistant gene subtypes from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistance gene families in said enriched nucleic acid sample, wherein identification of the one or more antibiotic resistant gene subtypes indicates that the patient has been colonized or infected with a multi-drug resistant bacterial organism

[00012] Also disclosed are surveillance methods to establish source of the infection or colonization, comprising by extracting a nucleic acid sample from a sample collected from the environment; amplifying the antibiotic resistant genes KPC, NDM, OXA, VIM, IMP, CTX-M and VanA and optionally one or more of IMI, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC, CMY, MIR, ACT, DHA, MOX, FOX, or CFE by contacting the nucleic acid sample with one or more amplification primers that specifically hybridize with the each of the antibiotic resistant genes to provide an

enriched nucleic acid sample; detecting the presence of the antibiotic gene by contacting the nucleic acid sample with one or more detection primers that specifically hybridize with the each of the antibiotic resistant genes to in the enriched nucleic acid sample; wherein the presence of the antibiotic gene in the sample indicates the source of the infection or colonization.

[00013] The patient is at high risk for having a multi-drug resistant bacteria colonization or infection.

[00014] Contact precautions includes for example, isolating the patient to a quarantine area or ward, providing a private room for said patient, donning personal protective apparel upon entering the patient's room, limiting patient mobility, limiting or restricting access of non-colonized or non-infected patients or medical personnel to the patient, or providing dedicated patient care equipment.

[00015] Biological samples, include but are not limited to an anal swab, a rectal swab, a skin swab, nasal swab, wound swab, stool, blood, plasma, serum, urine, sputum, respiratory lavage, cerebrospinal fluid, bacteria culture or bacteria isolate, fungal culture or fungal isolate, virus culture or virus isolate.

[00016] The disclosure also provides kits for determining whether a patient is colonized or infected with a multi-drug resistant bacteria including a biological sample collection means; one or more primers that specifically hybridize with one or more antibiotic resistant genes selected from KPC, NDM, OXA, VIM, IMP, CTX-M and VanA and optionally one or more of IMI, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC, CMY, MIR, ACT, DHA, MOX, FOX, or CFE; a control sample; and an instruction, wherein the instruction classifies the patient as being colonized or infected with a multi-drug resistant bacteria when one or more of the antibiotic resistant genes are identified in the sample.

[00016a] Also disclosed is a kit for determining whether a patient is colonized or infected with a multi-drug resistant bacteria comprising: a biological sample collection means; one or more primers that specifically hybridizes to a substantially homologous region common to at least two antibiotic gene subtypes from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families; a control sample; and an instruction, wherein said instruction classifies said patient as being colonized or infected with a multi-drug resistant bacteria when at least one antibiotic gene subtype from each of the antibiotic resistant gene families are identified in the sample

[00016b] In yet another aspect, the present invention provides a kit for determining whether a patient is colonized or infected with a multi-drug resistant bacterium comprising: a biological sample collection means; a plurality of sets of primers, wherein each set of primers specifically hybridizes to a substantially identical region common to at least two antibiotic resistant gene subtypes within one of each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families; a control nucleic acid sample; and an instruction, wherein said instruction classifies said patient as being colonized or infected with a multi-drug resistant bacterium when at least one antibiotic resistant gene subtype from each of the antibiotic resistant gene families is identified in the biological sample.

[00017] Also disclosed is method for implementation by one or more data processors forming part of at least one computing system by receiving, by at least one data processor, data characterizing test results of biological specimens for a patient or group of patients, wherein the test results characterize an outcome of a nucleic acid sample contacted with one or more primers that specifically hybridize with one or more antibiotic resistant genes selected from KPC, NDM, OXA, VIM, IMP, CTX-M and VanA and optionally one or more of IMI, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC,

CMY, MIR, ACT, DHA, MOX, FOX, or CFE; and generating, using at least one data processor and the received data, a database of test results for the patient or group of patients.

[00017a] Also disclosed is a method for implementation by one or more data processors forming part of at least one computing system comprising: receiving, by at least one data processor, data characterizing test results of biological specimens for a patient or group of patients, wherein the test results characterize an outcome of a nucleic acid sample contacted with one or more primer that specifically hybridizes to a substantially homologous region common to at least two antibiotic gene subtypes from each of the KPC, NDM, OXA, VIM, IMP, CTX-M and VanA; antibiotic resistant gene families; and generating, using at least one data processor and the received data, a database of test results for the patient or the group of patients.

[00018] In further examples the method further includes aggregating the database of test results with additional individual patient health data, patient group demographic data, healthcare institution demographic data, regional, national, or global geographic healthcare data.

[00019] The method further includes establishing, using the database of test results, colonization or infection rates and trends.

[00020] Optionally, the method further includes monitoring, using the database of test results, Hospital Acquired Infection (HAI) and death rates, CRE infection or death rates, ESBL infection or death rates.

[00021] Optionally, the method further includes establishing or augmenting, using the database of test results, treatment guidelines and policies, infection control procedures, or healthcare economic and risk management policies.

[00022] In yet a further example the method further includes computing, using the database of test results, baseline MDRO infection rates for a hospital and measuring the rate against a regional or national rate.

[00023] In other example the method further includes comprising augmenting treatment procedures, using the database of test results, to lower patient Length of Stay (LOS) in a healthcare institution, to lower overall costs, to lower patient death rates due to MDRO, to lower CMS penalties for HAIs, and to lower risk of legal settlements due to wrongful death claims.

[00024] The database of test results further includes an Electronic Health Record (EHR) or Laboratory Information Management System (LIMS).

[00025] Computer program products are also described that comprise non-transitory computer readable media storing instructions, which when executed by at least one data processors of one or more computing systems, causes at least one data processor to perform operations herein. Similarly, computer systems are also described that may include one or more data processors and a memory coupled to the one or more data processors. The memory may temporarily or permanently store instructions that cause at least one processor to perform one or more of the operations described herein. In addition, methods can be implemented by one or more data processors either within a single computing system or distributed among two or more computing system.

[00025a] Also disclosed is a surveillance method to identify a source of an infection or colonization, comprising: obtaining a nucleic acid sample from a sample collected from the environment; amplifying at least one antibiotic gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting the nucleic acid sample with one or more amplification primers that specifically hybridizes to a substantially homologous region common to at least two antibiotic gene subtypes in each antibiotic resistant gene family to provide an enriched nucleic acid sample; and detecting the presence of the at least one antibiotic gene subtype from each antibiotic resistant gene family by contacting the enriched nucleic acid sample with one or more detection primer that specifically hybridizes to the substantially homologous region or to another substantially homologous region common to at least two antibiotic gene subtypes in each antibiotic resistant gene family; wherein the presence of the at least one antibiotic gene subtype from each antibiotic resistant gene family in the enriched nucleic acid sample identifies the source of the infection or colonization.

[00025b] In yet another aspect, the present invention provides a surveillance method to identify a source of a multi-drug resistant bacterial infection or colonization, comprising: obtaining a nucleic acid sample from a sample collected from the environment; amplifying at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting the nucleic acid sample with a plurality of sets of amplification primers, wherein each set of amplification primers specifically

hybridizes to a substantially identical region common to at least two antibiotic gene subtypes within one of the antibiotic resistant gene families to provide an enriched nucleic acid sample, wherein the nucleic acid sample is contacted by an internal amplification control nucleic acid prior to amplification; and detecting the presence of the at least one antibiotic resistant gene subtype from each antibiotic resistant gene family by contacting the enriched nucleic acid sample with a plurality of sets of detection primers, wherein each set of detection primers specifically hybridizes to the substantially identical region or to another substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families; wherein the presence of the at least one antibiotic resistant gene subtype from each antibiotic resistant gene family in the enriched nucleic acid sample identifies the source of the infection or colonization.

Claims

We claim:

1. A method of screening a patient for multi-drug resistant bacterial colonization or infection, the method comprising:
 - isolating a nucleic acid sample from a biological sample obtained from the patient;
 - amplifying at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting the nucleic acid sample with a plurality of sets of amplification primers, wherein each set of amplification primers specifically hybridizes to a substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families to provide an enriched nucleic acid sample, wherein the nucleic acid sample is contacted by an internal amplification control nucleic acid prior to amplification;
 - detecting the presence of the at least one antibiotic resistant gene subtype by contacting the enriched nucleic acid sample with a plurality of sets of detection primers, wherein each set of detection primers specifically hybridizes to the substantially identical region or to another substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families, wherein said contacting results in amplification of the substantially identical region or amplification of another substantially identical region common to at least two antibiotic resistant gene subtypes within each antibiotic resistant gene family;
 - classifying the patient as having a multi-drug resistant bacterial colonization or infection when at least one antibiotic resistant gene subtype from each of the antibiotic resistant gene families is identified in the enriched nucleic acid sample; and
 - providing a contact precautions recommendation for the patient having a multi-drug resistant bacterial colonization or infection.
2. The method of claim 1, further comprising:

amplifying at least one antibiotic resistant gene subtype from one or more of the IML, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC, CMY, MIR, ACT, DHA, MOX, FOX, SIM, or CFE antibiotic resistant gene family by contacting the nucleic acid sample with a plurality of sets of amplification primers, wherein each set of amplification primers specifically hybridizes to a substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families to provide a further enriched nucleic acid sample; and

detecting the presence of the at least one antibiotic resistant gene subtype by contacting the further enriched nucleic acid sample with a plurality of sets of detection primers, wherein each set of detection primers specifically hybridizes to the substantially identical region or to another substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families.

3. The method of claim 1, wherein the contact precaution recommendation includes one or more of the following: isolating the patient to a quarantine area or ward, providing a private room for said patient, donning personal protective apparel upon entering the patient's room, limiting patient mobility, limiting or restricting access of non-colonized or non-infected patients or medical personnel to the patient, or providing dedicated patient care equipment.
4. The method of claim 1, wherein the biological sample is an anal swab, a rectal swab, a skin swab, a nasal swab, a wound swab, stool, blood, plasma, serum, urine, sputum, respiratory lavage, cerebrospinal fluid, a bacterial culture, a bacterial isolate, a fungal culture, a fungal isolate, a virus culture, or a virus isolate.
5. The method of claim 1, wherein said patient is at high risk for having a multi-drug resistant bacteria colonization or infection.
6. The method of claim 1, further comprising providing a treatment recommendation for said patient wherein

when at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, and IMP antibiotic resistant gene families is detected, recommending that the patient does not receive a carbapenem antibiotic;

when at least one antibiotic resistant gene subtype from the CTX-M antibiotic resistant gene family is detected, recommending that the patient does not receive a cephalosporin antibiotic; or

when at least one antibiotic resistant gene subtype from the VanA antibiotic resistant gene family is detected, recommending that the patient does not receive vancomycin.

7. The method of claim 2, further comprising providing a treatment recommendation for said patient wherein

when at least one antibiotic resistant gene subtype from one or more of the KPC, NDM, OXA, VIM, IMP, SME, SFC, IMI, NMC, or CcrA antibiotic resistant gene family is detected, recommending that the patient does not receive a carbapenem antibiotic;

when at least one antibiotic resistant gene subtype from one or more of the CTX-M, PER, VEB, GES, BES, SFO, or TLA antibiotic resistant gene family is detected, a TEM antibiotic gene subtype with amino acid substitution E104K, R164H, R164S, R164C, G238S, or E240K is detected, or an SHV antibiotic gene subtype with amino acid substitution G156D, G238S, or E240K, is detected, recommending that the patient does not receive a cephalosporin antibiotic;

when at least one antibiotic resistant gene subtype from one or more of the ACC, MOX, CMY, CFE, ACT, DHA, or FOX antibiotic resistant gene family is detected, recommending that the patient does not receive a beta-lactamase inhibitor-beta-lactam combination; or

when at least one antibiotic resistant gene subtype from the VanA antibiotic resistant gene family is detected, recommending that the patient does not receive vancomycin.

8. The method of claim 1, further comprising testing the biological sample to identify the phenotype of the multi-drug resistant organism.

9. The method of claim 1, further comprising culturing the biological sample and confirming drug resistance.
10. A method of making a treatment recommendation for a subject known to be or suspected of being colonized with or having a bacterial infection comprising:
- isolating a nucleic acid sample from a biological sample obtained from the patient;
 - amplifying at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting the nucleic acid sample with a plurality of sets of amplification primers, wherein each set of amplification primers specifically hybridizes to a substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families to provide an enriched nucleic acid sample, wherein the nucleic acid sample is contacted by an internal amplification control nucleic acid prior to amplification;
 - detecting the presence of the at least one antibiotic resistant gene subtype by contacting the enriched nucleic acid sample with a plurality of sets of detection primers, wherein each set of detection primers specifically hybridizes to the substantially identical region or to another substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families, wherein said contacting results in amplification of the substantially identical region or amplification of another substantially identical region common to at least two antibiotic resistant gene subtypes within each antibiotic resistant gene family; and
 - (i) recommending that the patient does not receive a carbapenem antibiotic when at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, and IMP antibiotic resistant gene families is detected;
 - (ii) recommending that the patient does not receive a cephalosporin antibiotic when at least one antibiotic resistant gene subtype from the CTX-M antibiotic resistant gene family is detected; or

(iii) recommending that the patient does not receive vancomycin when at least one antibiotic resistant gene subtype from the VanA antibiotic resistant gene family is detected.

11. The method of claim 10, further comprising amplifying and detecting at least one antibiotic resistant gene subtype from one or more of the IMI, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC, CMY, MIR, ACT, DHA, MOX, FOX, SIM, or CFE antibiotic resistant gene family, wherein

recommending that the patient does not receive a carbapenem antibiotic when at least one antibiotic resistant gene subtype from one or more of the SME, SFC, IMI, NMC, or CcrA antibiotic resistant gene family is detected;

recommending that the patient does not receive a cephalosporin antibiotic when at least one antibiotic resistant gene subtype from one or more of the PER, VEB, GES, BES, SFO, or TLA antibiotic resistant gene family is detected, a TEM antibiotic gene subtype with amino acid substitution E104K, R164H, R164S, R164C, G238S, or E240K is detected, or an SHV antibiotic gene subtype with amino acid substitution G156D, G238S or E240K is detected; or

recommending that the patient does not receive a beta-lactamase inhibitor-beta-lactam combination when at least one antibiotic resistant gene subtype from one or more of the ACC, MOX, CMY, CFE, ACT, DHA, or FOX antibiotic resistant gene family is detected.

12. A method of making a treatment recommendation for an asymptomatic subject comprising:

isolating a nucleic acid sample from a biological sample obtained from the subject;

amplifying at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting the nucleic acid sample with a plurality of sets of amplification primers, wherein each set of amplification primers specifically hybridizes to a substantially identical region common to at least two antibiotic resistant gene subtypes within one of

the antibiotic resistant gene families to provide an enriched nucleic acid sample, wherein the nucleic acid sample is contacted by an internal amplification control nucleic acid prior to amplification;

detecting the presence of the at least one antibiotic resistant gene subtype by contacting the enriched nucleic acid sample with a plurality of sets of detection primers, wherein each set of detection primers specifically hybridizes to the substantially identical region or to another substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families, wherein said contacting results in amplification of the substantially identical region or amplification of another substantially identical region common to at least two antibiotic resistant gene subtypes within each antibiotic resistant gene family; and

(i) recommending that the subject does not receive a carbapenem antibiotic when at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, and IMP antibiotic resistant gene families is detected;

(ii) recommending that the subject does not receive a beta-lactam antibiotic when at least one antibiotic resistant gene subtype from the CTX-M antibiotic resistant gene family is detected; or

(iii) recommending that the subject does not receive vancomycin when at least one antibiotic resistant gene subtype from the VanA antibiotic resistant gene family is detected.

13. The method of claim 12, further comprising amplifying and detecting at least one antibiotic resistant gene subtype from one or more of the IMI, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC, CMY, MIR, ACT, DHA, MOX, FOX, SIM, or CFE antibiotic resistant gene family wherein

recommending that the subject does not receive a carbapenem antibiotic when at least one antibiotic resistant gene subtype from one or more of the SME, SFC, IMI, NMC, or CcrA antibiotic resistant gene family is detected;

recommending that the subject does not receive a cephalosporin antibiotic when at least one antibiotic resistant gene subtype from one or more of the PER, VEB, GES, BES, SFO, or TLA antibiotic resistant gene family is detected, a TEM antibiotic gene

subtype with amino acid substitution E104K, R164H, R164S, R164C, G238S, or E240K is detected, or an SHV antibiotic gene subtype with amino acid substitution G156D, G238S, or E240K is detected; or

recommending that the subject does not receive a beta-lactamase inhibitor-beta-lactam combination when at least one antibiotic resistant gene subtype from one or more of the ACC, MOX, CMY, CFE, ACT, DHA, or FOX antibiotic resistant gene family is detected.

14. The method of claim 12, wherein the biological sample is an anal swab, a rectal swab, a skin swab, a nasal swab, a wound swab, stool, blood, plasma, serum, urine, sputum, respiratory lavage, cerebrospinal fluid, a bacterial culture, a bacterial isolate, a fungal culture, a fungal isolate, a virus culture, or a virus isolate.
15. A method of identifying the emergence of a multi-drug resistant organism in a population of subjects, the method comprising:
 - isolating a plurality of nucleic acid samples from a plurality of biological samples obtained from a plurality of subjects in the population of subjects;
 - amplifying at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting each nucleic acid sample with a plurality of sets of amplification primers, wherein each set of amplification primers specifically hybridizes to a substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families to provide a plurality of enriched nucleic acid samples, wherein each nucleic acid sample is contacted by an internal amplification control nucleic acid prior to amplification; and
 - identifying one or more antibiotic resistant gene subtypes from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families in the plurality of the enriched nucleic acid samples,
 - wherein identification of the one or more antibiotic resistant gene subtypes in the plurality of the enriched nucleic acid samples indicates the emergence of a multi-drug resistant organism in the population of subjects.

16. The method of claim 15, further comprising identifying one or more antibiotic gene subtypes from at least one of the IMI, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC, CMY, MIR, ACT, DHA, MOX, FOX, SIM, or CFE antibiotic resistant gene family.
17. A method of screening a patient for a multi-drug resistant bacterial colonization or infection, the method comprising:
- isolating a nucleic acid sample from a biological sample obtained from the patient such that the nucleic acid sample is substantially free of protein, cellular debris, and /or PCR inhibitors;
 - amplifying at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting the nucleic acid sample with a plurality of sets of amplification primers, wherein each set of amplification primers specifically hybridizes to a substantially identical region common to at least two antibiotic gene subtypes within one of the antibiotic resistant gene families to provide an enriched nucleic acid sample, wherein the nucleic acid sample is contacted by an internal amplification control nucleic acid prior to amplification; and
 - identifying one or more antibiotic resistant gene subtypes from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistance gene families in said enriched nucleic acid sample,
 - wherein identification of the one or more antibiotic resistant gene subtypes indicates that the patient has been colonized or infected with a multi-drug resistant bacterial organism.
18. The method of claim 17, further comprising identifying one or more antibiotic resistant gene subtypes from at least one of the IMI, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC, CMY, MIR, ACT, DHA, MOX, FOX, SIM, or CFE antibiotic resistant gene family.

19. The method of claim 17, further comprising

recommending that the patient does not receive a carbapenem antibiotic when at least one antibiotic resistant gene subtype from the KPC, NDM, OXA, VIM, or IMP antibiotic resistant gene family is detected;

recommending that the patient does not receive a beta-lactam antibiotic when at least one antibiotic resistant gene subtype from the CTX-M antibiotic resistant gene family is detected; or

recommending that the patient does not receive vancomycin when at least one antibiotic resistant gene subtype from the VanA antibiotic resistant gene family is detected.

20. The method of claim 17, further comprising identifying one or more antibiotic resistant gene subtypes from at least one of the IMI, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC, CMY, MIR, ACT, DHA, MOX, FOX, SIM, or CFE antibiotic resistant gene family, wherein

recommending that the patient does not receive a carbapenem antibiotic when at least one antibiotic resistant gene subtype from the SME, SFC, IMI, NMC, or CcrA antibiotic resistant gene family is detected;

recommending that the patient does not receive a cephalosporin antibiotic when at least one antibiotic resistant gene subtype from the PER, VEB, GES, BES, SFO, or TLA antibiotic resistant gene family is detected, a TEM antibiotic resistant gene subtype with amino acid substitution E104K, R164H, R164S, R164C, G238S, or E240K is detected, or an SHV antibiotic resistant gene subtype with amino acid substitution G156D, G238S, or E240K is detected; or

recommending that the patient does not receive a beta-lactamase inhibitor-beta-lactam combination when at least one antibiotic gene resistant subtype from the ACC, MOX, CMY, CFE, ACT, DHA, or FOX antibiotic resistant gene family is detected.

21. A kit for determining whether a patient is colonized or infected with a multi-drug resistant bacterium comprising:

a biological sample collection means;

a plurality of sets of primers, wherein each set of primers specifically hybridizes to a substantially identical region common to at least two antibiotic resistant gene subtypes within one of each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families;

a control nucleic acid sample; and

an instruction, wherein said instruction classifies said patient as being colonized or infected with a multi-drug resistant bacterium when at least one antibiotic resistant gene subtype from each of the antibiotic resistant gene families is identified in the biological sample.

22. The kit of claim 21, further comprising one or more sets of primers, wherein each set of primers specifically hybridizes to a substantially identical region common to at least two antibiotic resistant gene subtypes within one or more of the IMI, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC, CMY, MIR, ACT, DHA, MOX, FOX, SIM, or CFE antibiotic resistant gene family.
23. A surveillance method to identify a source of a multi-drug resistant bacterial infection or colonization, comprising:
- obtaining a nucleic acid sample from a sample collected from the environment;
 - amplifying at least one antibiotic resistant gene subtype from each of the KPC, NDM, OXA, VIM, IMP, CTX-M, and VanA antibiotic resistant gene families by contacting the nucleic acid sample with a plurality of sets of amplification primers, wherein each set of amplification primers specifically hybridizes to a substantially identical region common to at least two antibiotic gene subtypes within one of the antibiotic resistant gene families to provide an enriched nucleic acid sample, wherein the nucleic acid sample is contacted by an internal amplification control nucleic acid prior to amplification; and
 - detecting the presence of the at least one antibiotic resistant gene subtype from each antibiotic resistant gene family by contacting the enriched nucleic acid sample with a plurality of sets of detection primers, wherein each set of detection primers specifically

hybridizes to the substantially identical region or to another substantially identical region common to at least two antibiotic resistant gene subtypes within one of the antibiotic resistant gene families;

wherein the presence of the at least one antibiotic resistant gene subtype from each antibiotic resistant gene family in the enriched nucleic acid sample identifies the source of the infection or colonization.

24. The method of claim 23, wherein at least one antibiotic resistant gene subtype from one or more of the IMI, SME, GIM, SPM, NMC, SFC, SHV, TEM, BEL, VEB, GES, PER, SFO, BES, TLA, ACC, CMY, MIR, ACT, DHA, MOX, FOX, SIM, or CFE antibiotic resistant gene family is amplified and detected.