United States Patent	12385102
Kind Code	B2
Date of Patent	August 12, 2025
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Assays and methods for determining microbial resistance

Abstract

Assays and methods for detecting resistance to beta-lactam antibiotics including detection of multiple β-lactamase family specific gene targets by polymerase chain reaction or microarray. One or more kits including primers and/or probes for identification of β-lactamase genes selected from the group consisting of one or more of the following: MOX-like, FOX-like, ACC-like, ACT/MIR-like, CMY-2-like, DHA-like, CTX-M-14-like, CTX-M-15-like, VIM-like, NDM-like, IMP-like, KPC-like, and OXA-48-like, OXA-51-like, OXA-143-like, OXA-58-like, OXA-23-like, OXA-24/40-like, TEM-like, and SHV-like. A kit may also include one or more primers and/or probes for the identification a non-beta lactamase gene family which confers antibiotic resistance, such as the MCR-1 gene.

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Appl. No.: **18/332590**

Filed: **June 09, 2023**

Prior Publication Data

Document Identifier	Publication Date
US 20240150850 A1	May. 09, 2024

Related U.S. Application Data

division parent-doc US 16310074 US 11708614 WO PCT/US2017/037700 20170615 child-doc US 18332590
us-provisional-application US 62350457 20160615

Publication Classification

Int. Cl.: **C12Q1/689** (20180101); **C12Q1/6809** (20180101); **C12Q1/6837** (20180101); **C12Q1/6851** (20180101); **C12Q1/686** (20180101)

U.S. Cl.:

CPC **C12Q1/689** (20130101); **C12Q1/6809** (20130101); **C12Q1/6837** (20130101); **C12Q1/6851** (20130101); **C12Q1/686** (20130101); C12Q2545/101 (20130101); C12Q2600/106 (20130101); C12Q2600/16 (20130101); C12Q2600/166 (20130101)

Field of Classification Search

USPC: None

References Cited

U.S. PATENT DOCUMENTS

Patent No.	Issued Date	Patentee Name	U.S. Cl.	CPC
3687808	12/1971	Merigan et al.	N/A	N/A
4845205	12/1988	Huynh et al.	N/A	N/A
5130302	12/1991	Spielvogel et al.	N/A	N/A
5134066	12/1991	Rogers et al.	N/A	N/A
5175273	12/1991	Bischofberger et al.	N/A	N/A
5432272	12/1994	Benner	N/A	N/A
5457187	12/1994	Gmeiner et al.	N/A	N/A
5459255	12/1994	Cook et al.	N/A	N/A
5484908	12/1995	Froehler et al.	N/A	N/A
5502177	12/1995	Matteucci et al.	N/A	N/A
5525711	12/1995	Hawkins et al.	N/A	N/A
5539082	12/1995	Nielsen et al.	N/A	N/A
5552540	12/1995	Haralambidis	N/A	N/A
5587469	12/1995	Cook et al.	N/A	N/A
5594121	12/1996	Froehler et al.	N/A	N/A
5596091	12/1996	Switzer	N/A	N/A
5614617	12/1996	Cook et al.	N/A	N/A
5645985	12/1996	Froehler et al.	N/A	N/A
5681941	12/1996	Cook et al.	N/A	N/A
5714331	12/1997	Buchardt et al.	N/A	N/A
5719262	12/1997	Buchardt et al.	N/A	N/A
5750692	12/1997	Cook et al.	N/A	N/A
5763588	12/1997	Matteucci et al.	N/A	N/A
5830653	12/1997	Froehler et al.	N/A	N/A
6005096	12/1998	Matteucci et al.	N/A	N/A

6242223	12/2000	Hanson et al.	N/A	N/A
6893846	12/2004	Hanson et al.	N/A	N/A
6905848	12/2004	Hanson et al.	N/A	N/A
7045291	12/2005	Hanson et al.	N/A	N/A
7476520	12/2008	Hanson et al.	N/A	N/A
7521547	12/2008	Hanson et al.	N/A	N/A
9120840	12/2014	Janssen et al.	N/A	N/A
11708614	12/2022	Torres-Gonzalez et al.	N/A	N/A
2003/0219749	12/2002	Hanson et al.	N/A	N/A
2007/0248954	12/2006	Hanson	N/A	N/A
2009/0197275	12/2008	Boonyarantanakornkit et al.	N/A	N/A
2016/0085912	12/2015	Jones et al.	N/A	N/A
2019/0017774	12/2018	Vanek et al.	N/A	N/A

FOREIGN PATENT DOCUMENTS

Patent No.	Application Date	Country	CPC
104946764	12/2014	CN	N/A
1072679	12/2000	EP	N/A
10-1908870	12/2017	KR	N/A
97/12896	12/1996	WO	N/A
98/39352	12/1997	WO	N/A
99/06594	12/1998	WO	N/A
99/14226	12/1998	WO	N/A
2010/130882	12/2009	WO	N/A
2012/027302	12/2011	WO	N/A
2012/027302	12/2011	WO	N/A
2015/138991	12/2014	WO	N/A
2015/138991	12/2014	WO	N/A
2016/067047	12/2015	WO	N/A
2017/218789	12/2016	WO	N/A

OTHER PUBLICATIONS

Streck, Philisa ampC ID Kit Ad—CLP, Identify the threat of antibiotic resistant bacteria faster, 1 page (Sep. 2014). cited by applicant

Streck, Philisa ampC ID Kit JMD, Identify the threat of antibiotic resistant bacteria faster with the philisa (Registered) ampC ID kit, 1 page (Nov. 2014). cited by applicant

Streck, Philisa ampC ID Kit Poster Ad—CAP Today, Identify the threat of antibiotic resistant bacteria faster with the philisa (Registered) ampC ID kit, 1 page (Oct. 2014). cited by applicant

Streck, Streck ARM-D Kit, BETA-Lactamase (RUO), Ref. 350670-1 (Nov. 2017). cited by applicant

Streck, Streck ARM-D Kits (RUO) Data Acquisition & Analysis Guide—Bio-Rad CFX96 Touch (Trademark) Real-Time PCR Detection System. Ref. 880107-1 (Dec. 2017). cited by applicant

Torres et al., Detection of ESBLs, MBLs, KPCs, and plasmid-mediated ampC (Beta)-lactamase genes by multiplex real-time PCR, American Society for Microbiology (ASM) Microbe 2016 Conference, 12 pages. (Jun. 18, 2016). Poster presentation. cited by applicant

Torres et al., Rapid detection of OXA (Beta)-lactamases by multiplex real-time PCR, Streck research and development-molecular technology division, La Vista, NE, 1 (2011). cited by applicant

Torres et al., Rapid detection of plasmid-mediated ampC (Beta)-lactamase genes by multiplex real-time PCR, American Society for Microbiology (ASM) Microbe 2015 Conference, 1O pages (May

31, 2015). Poster presentation. cited by applicant
Torres et al., Rapid detection of plasmid-mediated ampC (Beta)-lactamase genes by multiplex real-time PCR, Streck, Research and development, Molecular technology division, 1 (2015). cited by applicant
Vandenbussche et al., A Tale of Tails: Dissecting the Enhancing Effect of Tailed Primers in Real-Time PCR, PLoS One, 11(10):e0164463 (2016). cited by applicant
Vazquez-Ucha et al., Activity of the (Beta)-Lactamase Inhibitor LN-1-255 against Carbapenem-Hydrolyzing Class D (Beta)-Lactamases from Acinetobacter baumannii, Antimicrobial Agents and Chemotherapy, 61(11):e01172-17 (2017). cited by applicant
Vázquez-Ucha et al et al., Activity of the B-Lactamase Inhibitor LN-1-255 against Carbapenem-Hydrolyzing Class D ß-Lactamases from Acinetobacter baumannii, Antimicrobial Agents and Chemotherapy, 61(11):e01172-17 (2017). cited by applicant
Yin et al., Novel Plasmid-Mediated Colistin Resistance Gene mcr-3 in *Escherichia coli*, mBIO, 8(3):e00543-17 (2017). cited by applicant
"Streck Arm-D Kits," Jun. 22, 2017. cited by applicant
Alao et al., Detection of mobilized colistin resistance (mcr) genes by multiplex real-time PCR: improving surveillance of an emerging threat, APHL, 1 (2019). cited by applicant
Alao et al., Improved methodology for detection of antibiotic resistance in gram-negative bacteria, European Congress of Clinical Microbiology and Infectious Diseases (ECCMID) Conference, 14 pages. (Apr. 24, 2018). Poster presentation. cited by applicant
Anandan et al., Structure of a lipid A phosphoethanolamine transferase suggests how conformational changes govern substrate binding, Proc. Nat. Acad. Sci. USA, 114(9):2218-2223 (2017). cited by applicant
Antunes et al., Acquired Class D ß-Lactamases, Antibiotics, 3(3):398-434 (2014). cited by applicant
Beck et al., Digital Droplet PCR for Rapid Quantification of Donor DNA in the Circulation of Transplant Recipients as a Potential Universal Biomarker of Graft Injury, Clinical chemistry, 59(12):1732-41 (2013). cited by applicant
Borowiak et al., Identification of a novel transposon-associated phosphoethanolamine transferase gene, mcr-5, conferring colistin resistance in d-tartrate fermenting *Salmonella enterica* subsp. enterica serovar Paratyphi B, J. Antimicrob Chemother, 72:3317-3324 (2017). cited by applicant
Carattoli et al., Novel plasmid-mediated colistin resistance mcr-4 gene in Salmonella and *Escherichia coli*, Italy 2013, Spain and Belgium 2015 to 2016, Euro Surveill, 22(31):30589 (2017). cited by applicant
Concise encyclopedia of polymer science and engineering, J. I. Kroschwitz Ed., John Wiley & Sons, 858-859 (1990). cited by applicant
Connelly et al., Antiobiotic Resistance Monitoring and Detection (ARM-D™) PCR Kits: ARM-D™ for b-Lactamase ID—Technical Note—Detection of ESBLs, MBLs, KPCs, and plasmid-mediated AmpCs, using the b-Lactamase ID kit to identify antiuobiotic resistance in Gram-negative pathogens, Biotechniques Rapid Dispatches, 57(6):317-318 (2014). cited by applicant
Cook, Medicinal chemistry of antisense oligonucleotides-future opportunities, Anti-Cancer Drug Design, 6(6):585-607 (1991). cited by applicant
Cossette et al., Rapid detection of OXA (Beta)-lactamases by multiplex real-time PCR, American Society for Microbiology (ASM) Microbe 2018 Conference, 8 pages (Jun. 8, 2018). Poster presentation. cited by applicant
Cussac et al., Reduction of the toxicity and mutagenicity of aziridine in mammalian cells harboring the *Escherichia coli* fpg gene, Nucleic Acids Research, 24(9):1742-1746 (1996). cited by applicant
Declaration of Nicole Quackenbush, dated Mar. 8, 2022, filed during the prosecution of U.S. Appl. No. 16/310,074. cited by applicant
Ebili et al., "Squirrel" Primer-Based PCR Assay for Direct and Targeted Sanger Sequencing of

Short Genomic Segments, J. Biomol. Tech., 28(3):97-110 (2017). cited by applicant
Englisch et al., Chemically Modified Oligonucleotides as Probes and Inhibitors, Angew. Chem. Int. Ed., 30(6):613-629 (1991). cited by applicant
Evans et al., OXA ß-Lactamases, Clinical Microbiology Reviews, 27(2):241-263 (2014). cited by applicant
Extended European Search Report dated Oct. 21, 2016; Application No. 15196213.1. cited by applicant
Freier et al., The ups and downs of nucleic acid duplex stability: structure-stability studies on chemically-modified DNA:RNA duplexes, Nucl. Acid. Res., 25:4429-4443 (1997). cited by applicant
Geyer et al., Development of a TaqMan multiplex PCR assay for detection of plasmid-mediated ampC ß-lactamase genes, J. Clin. Microbiol., 50(11):3722-5 (2012). cited by applicant
Hanson, One 20-minute multiplex assay for the detection of 10 targets including ampCs, ESBLs, MBLs, and KPCs using rapid PCR amplification, Center for research in anti-infectives and biotechnology creighton university omaha, NE, 19 (2014). cited by applicant
International Application No. PCT/US20/35422, International Preliminary Report on Patentability, mailed Dec. 9, 2021. cited by applicant
International Preliminary Report on Patentability for International Application No. PCT/US2014/047551 dated Dec. 10, 2015. cited by applicant
International Preliminary Report on Patentability from International Application No. PCT/US2012/036304 dated Aug. 16, 2013. cited by applicant
International Preliminary Report on Patentability from International Application No. PCT/US2017/37700, dated Dec. 27, 2018. cited by applicant
International Search Report and Written Opinion from International Application No. PCT/US20/35422 dated Nov. 13, 2020. cited by applicant
International Search Report and Written Opinion from International Application No. PCT/US2012/036304 dated Nov. 21, 2012. cited by applicant
International Search Report and Written Opinion from International Application No. PCT/US2017/037700 dated Dec. 21, 2017. cited by applicant
International Search Report and Written Opinion from International Application No. PCT/US2017/046537 dated Oct. 23, 2017. cited by applicant
Moland et al., Occurrence of Newer-Lactamases in Klebsiella pneumoniae Isolates from 24 U.S. Hospitals, Ant. Agen. Chem., 46(12):3837-3842 (2002). cited by applicant
Nielsen et al., Sequence-selective recognition of DNA by strand displacement with a thymine-substituted polyamide, Science, 254(5037):1497-1500 (1991). cited by applicant
Perez-Perez et al., Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR, J. Clin. Micro., 40(6):2153-2162 (2002). cited by applicant
Pitout et al., Phenotypic and Molecular Detection of CTX-M—Lactamases Produced by *Escherichia coli* and Klebsiella spp, J. Clin. Micro., 42(12):5715-5721 (2004). cited by applicant
Poirel et al., Diversity, epidemiology, and genetics of class D beta-lactamases, Antimicrobial Agents and Chemotherapy, 54(1):24-38 (2010). cited by applicant
Sanghvi, Chapter 15, Antisense research and applications, Ed. S. T. Crooke and B. Lebleu, CRC Press, 289-302 (1993). cited by applicant
Silbert, Evaluation of different tests to screen and identify carbapenemase-production bacteria strains, Microbiology, 43 (2019). cited by applicant
Sobansky et al., Development of Accurate and Reliable Full-Process Controls that Mimic Patient Samples for Molecular Diagnostic Testing, Poster Presentation ASM Microbe (2017). cited by applicant
Streck, Philisa ampC ID Kit Ad—CAP Today, Identify the threat of antibiotic resistant bacteria faster, 1 page (May 2014). cited by applicant

Favaro et al., Multiplex real-time PCR probe-based for identification of strains producing: OXA48, VIM, KPC and NDM, World Journal of Microbiology and Biotechnology, 30(1): 2995-3001(2014). cited by applicant
Kaase et al., Detection of Carbapenemases in Enterobacteriaceae by a Commercial Multiplex PCR, Journal of Clinical Microbiology, 50(9): 3115-3118(2012). cited by applicant
Hernandez et al., Stability study of (Beta)-lactamase detection from gram-negative bacilli directly from positive blood cultures using two commercially available PCR kits, American Society for Microbiology (ASM) Microbe 2018 Conference, 8 pages. (Jun. 8, 2018). Poster presentation. cited by applicant
Quickgene, Series Application Guide, Genomic DNA extraction from Pseudomonas aeruginosa. 37 (Jun. 2023). cited by applicant
The Streck ARM-D (Registered) kits are multiplex real-time PCR kits for the detection of clinically-relevant(Beta)-lactamase genes, Product overview, 27 pages (Jan. 2021). cited by applicant
Database EMBL [Online], Citrobacter freundii strain 90757 class C beta-lactamase CMY-39 gene, complete cds, XP002772843, retrieved from EBI accession No. EM STD:HM565135 Database accession No. HM565135 (2010). cited by applicant
Database EMBL [Online], Citrobacter freundii strain W704 AmpR transcriptional regulator (ampR) and AmpC beta-lactamase CMY-80 (blaCMY-80) genes, complete eds; and outer membrane lipoprotein Blc (blc) gene, partial cds, XP002772846, retrieved from EBI accession No. EM STD:JQ733577 Database accession No. JQ733577 (2012). cited by applicant
Database EMBL [Online], Citrobacter freundii strain W811 transcriptional regulator AmpR (ampR) gene, partial eds; beta-lactamase AmpC (blaCMY-86) gene, complete eds; and outer membrane lipoprotein Blc (blc) gene, partial cds, XP002772845, retrieved from EBI accession No. EM STD: KJ207204 Database accession No. KJ207204 (2014). cited by applicant
Database EMBL [Online], *Escherichia coli* strain 2011/34/01 AmpC beta-lactamase (blaCMY-108) gene, complete cds, XP002772844, retrieved from EBI accession No. EM STD: KF564648 Database accession No. KF564648 (2014). cited by applicant
Database Geneseq [online] Apr. 12, 2012 (Apr. 12, 2012) “Antibiotic resistance gene sequence, Seq ID 227”, Apr. 12, 2012, Accessin No. AZT75073. cited by applicant
Database Geneseq [Online], Antibiotic resistance gene CFE-1 forward PCR primer Seq ID 120, XP002772841 retrieved from EBI accession No. GSN:BCE41068 Database accession No. BCE41068 (2015). cited by applicant
Database Geneseq [Online], *Escherichia coli* beta-lactamase gene fragment, Seq ID 7, XP002772847, retrieved from EBI accession No. GSN:BCM28931 Database accession No. BCM28931 (2016). cited by applicant
Integrated DNA Technologies, Better PCR probes: A second quencher lowers backrgound, increasing signal detection, 2021. (Year: 2021). cited by applicant
Alao et al., Detection of mobilized colistin resistance (mer) genes by multiplex real-time PCR: improving surveillance of an emerging threat, Association of Public Health Laboratories (APHL) Conference, 6 pages (Jun. 4, 2019). Poster presentation. cited by applicant
Alao et al., Improved methodology for detection of antibiotic resistance in gram-negative bacteria, Streck research and development-molecular technology division, La Vista, NE, 1 page (2018). cited by applicant
Torres et al., Detection of ESBLs, MBLs, KPCs, and plasmid-mediated ampC (Beta)-lactamase genes by multiplex real-time PCR, Streck research and development, molecular technology division, Omaha, NE, 1 page (2016). cited by applicant

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Background/Summary

CROSS-REFERENCE TO RELATED APPLICATIONS (1) This application is a Divisional of U.S. application Ser. No. 16/310,074, filed Dec. 14, 2018, now U.S. Pat. No. 11,708,614, issued Jul. 25, 2023, which is a U.S. National Phase of PCT/US17/37700, filed Jun. 15, 2017, which claims the priority benefit under 35 U.S.C. § 119(e) of U.S. Provisional Patent Application No. 62/350,457, filed Jun. 15, 2016, which are incorporated herein by reference in their entirety.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY
(1) The Sequence Listing, which is a part of the present disclosure, is submitted concurrently with the specification as a text file. The name of the text file containing the Sequence Listing is "50035A_SubSeqListing.xml", which was created on Jun. 8, 2023 and is 403,656 bytes in size. The subject matter of the Sequence Listing is incorporated herein in its entirety by reference.
FIELD
(2) The present teachings relate to assays and methods for detecting resistance to antibiotics. The present teachings provide for the detection of family specific gene targets including AmpC β-lactamases, metallo-β-lactamases, carbapenemases, and extended-spectrum β-Lactamases by multiplex real-time polymerase chain reaction.
BACKGROUND
(3) Bacterial resistance to antibiotics is a major public health issue. This resistance not only presents severe limitations to the ability to control and treat infection, but it also is difficult to identify and characterize in the laboratory. The significant increase in the resistance of pathogenic bacteria over the last 20 years, leads to extended periods of hospitalization, high morbidity and high mortality rates.
(4) Enzymatic inactivation is the most common cause of resistance in terms of number of species and of antibiotics involved. As an example, β-lactamases are enzymes expressed by some bacteria. Such enzymes are capable of hydrolyzing the C—N bond of the β-lactam ring structure of a β-lactam antibiotic, effectively inactivating the antibiotic. Despite the existence of several β-lactamase inhibitors, the constant exposure of strains to antibiotics results in constant evolution of β-lactamases.
(5) As a result, it becomes essential to be able to identify such resistant microorganisms and their resistance mechanisms as quickly as possible. Typically, biological samples can be tested for antibiotic resistance, but many test protocols are time consuming and/or limited in the types of resistance they are able to identify. It would therefore be beneficial to provide a test protocol for the simplified identification of resistance for all major β-lactamases.
(6) One approach to the identification of β-lactamases has been to employ oligonucleotide primers specific for nucleic acid characteristic of certain β-lactamases with polymerase chain reaction to identify nucleic acid characteristics of family specific β-lactamase enzymes in samples. See for example, U.S. Pat. Nos. 6,893,846 and 7,476,520, incorporated by reference herein. Another approach has been to employ oligonucleotide primers specific for nucleic acid characteristic of certain AmpC β-lactamases with multiplex polymerase chain reaction to detect the presence or absence of an AmpC β-lactamase gene and to identify nucleic acid characteristic of AmpC β-lactamase genes in samples. Multiplex polymerase chain reaction refers to the use of polymerase chain reaction to amplify several different DNA sequences simultaneously in single or multiple reactions. See for example, U.S. Pat. Nos. 7,045,291 and 7,521,547 incorporated by reference

herein.
(7) However, such primers have been limited with regards to the number of β-lactamase gene families or the number of gene targets that may be identified. Furthermore, such primers have been employed mainly with conventional polymerase chain reaction, which typically requires agarose gels to detect and analyze the PCR product(s). The use of agarose gel detection methods based on size discrimination may lead to poor resolution and difficulty in interpreting the data. Conventional polymerase chain reaction also lacks the sensitivity to detect endpoint variability from sample to sample and may not be automated. Real-time polymerase chain reaction allows for monitoring of reaction products as they are formed.
(8) Detection of β-lactamases using real-time polymerase chain reaction and a single primer set may be limited to detection of a single β-lactamase gene family. See for example, United States Patent Application 2007/0248954 incorporated by reference herein. Multiplex real-time polymerase chain reaction has been designed for the identification of many AmpC β-lactamases simultaneously. See Geyer C N, Reisbig M D, Hanson N D. Development of a TaqMan® Multiplex PCR Assay for Detection of Plasmid-Mediated AmpC β-lactamase Genes. *Journal of clinical microbiology.* 2012 Aug. 15:JCM-02038. The primer/probe combinations in this study, however, have been directed only to AmpC β-lactamases and are limited in the number of gene targets that may be identified.
(9) Multiple factors such as primer and probe design, reaction conditions, and enzyme selection must all be considered when designing a working polymerase chain reaction. This complexity is compounded in multiplex PCR, in which multiple targets are detected simultaneously in the same tube. Balancing the concentrations of primers, probes, and control vectors provided as composite "multiplex PCR" mixes for an assay is a challenging aspect. It is extremely difficult to balance these ratios, as a change of concentration for any of these reagents, corresponding to just one of the genetic targets, may adversely affect detection of any other multiplex target in the reaction mix. If these concentrations are not balanced, one could expect a reduction in efficiency, sensitivity, and specificity. This would reduce confidence in the effectiveness of the assay to correctly identify the gene families identified with the described kits.
(10) Therefore, there is a significant amount of time and technical know-how required to develop these assays into a reliable method. For example, the PCR master mixture, with DNA polymerase, is a customized formulation that permits the final assay to work. Concentrations of DNA polymerase and magnesium may have to be adjusted. The specific concentrations and ranges surrounding DNA polymerase and magnesium are required for the assay to work successfully. In addition to determining concentrations for all reagents, a PCR cycling protocol must be identified that is compatible with all reaction conditions and facilitates real-time multiplex polymerase chain reaction.
(11) Accurate and rapid detection of antibiotic resistance is essential for surveillance, epidemiologic tracking, patient therapy, and infection control. Thus, a multiplex PCR based diagnostic assay should provide comprehensive genotypic characterization of β-lactamases and be versatile as well as providing rapid results. The present teachings make it possible to test a sample for the presence of antibiotic resistant microorganisms by identifying any of the major β-lactamases in one test. The present teachings provide for the detection of multiple family-specific β-lactamase gene targets, including but not limited to metallo-β-lactamases, carbapenemases, extended-spectrum β-Lactamases, ampC chromosomal and/or plasmid-mediated AmpC β-lactamases, by multiplex real-time polymerase chain reaction.
(12) The present teachings provide for a kit or kits including one or more primers and/or probes for identification of β-lactamase genes selected from the group consisting of one or more of the following: MOX-like, FOX-like, ACC-like, ACT/MIR-like, CMY-2-like, DHA-like, CTX-M-14-like, CTX-M-15-like, VIM-like, NDM-like, IMP-like, KPC-like, and OXA-48-like, OXA-51-like, OXA-143-like, OXA-58-like, OXA-23-like, OXA-24/40-like, TEM-like, and SHV-like. The kits or

kits of the present teachings may provide control material for the aforementioned β-lactamase genes. The present teachings provide one or more of the following: primers, probes, controls, assay process and detection strategy for one or more of the following β-lactamases: extended-spectrum β-lactamases (ESBLs), metallo-β-lactamases (MBLs), carbapenem-resistant enterobacteriaceaes (CREs), and serine-dependent carbapenemases and plasmid-mediated ampC β-lactamases. A kit may also include one or more primers and/or probes for the identification of mobilized colistin-resistant (MCR) genes, a non-beta lactamase gene family that confers antibiotic resistance. The present teachings provide multiplex PCR assays which may test for any combination of these or are directed towards identification of a specific group. The present teachings provide assays with improved clinical sensitivity and analytical specificity of detection. The primer, probes, and control DNA sequences of the present teachings provide both an analytical and commercial advantage as they permit enhanced screening capabilities for detection of a larger number of genetic variants associated with genes conferring resistance to antibiotics in Gram-negative bacteria.

SUMMARY

(13) The present teachings provide a kit including one or more primers and/or probes for the identification by polymerase chain reaction, microarray, NGS-based target enrichment, and/or mass spectrometric characterization of one or more β-lactamase genes selected from the group consisting of: CMY, CTX-M, OXA, IMP, VIM, DHA, KPC, MOX, ACC, FOX, EBC, NDM, TEM, and SHV. The present teachings provide for one or more kits including primers and/or probes for identification of β-lactamase genes selected from the group consisting of one or more of the following: MOX-like, FOX-like, ACC-like, EBC-like, CMY-2-like, DHA-like, CTX-M-14-like, CTX-M-15-like, VIM-like, NDM-like, IMP-like, KPC-like, and OXA-48-like, OXA-51-like, OXA-143-like, OXA-58-like, OXA-23-like, OXA-24/40-like, TEM-like, and SHV-like. A kit may also include one or more primers and/or probes for the identification of a non-beta lactamase gene family which confers antibiotic resistance. A kit may include one or more primers and/or probes for the identification by polymerase chain reaction or microarray of MCR gene variants. Primers and probes may also be made compatible with next-generation sequencing and mass spectrometry.

Description

BRIEF DESCRIPTION OF DRAWINGS

(1) FIG. **1** depicts an amplification plot of an exemplary mix 1 of a kit including ampC gene targets.

(2) FIG. **2** depicts an amplification plot of an exemplary mix 2 of a kit including ampC gene targets.

(3) FIG. **3** depicts an amplification plot of an exemplary mix 1 of a kit including β-lactamase gene targets.

(4) FIG. **4** depicts an amplification plot of an exemplary mix 2 of a kit including β-lactamase gene targets.

(5) FIG. **5** depicts an amplification plot of an exemplary mix 3 of a kit including β-lactamase gene targets.

(6) FIG. **6** depicts an amplification plot of an exemplary internal control mix of a kit including MCR gene targets.

(7) FIG. **7** depicts an amplification plot of an exemplary mix 1 of a kit including OXA gene targets.

(8) FIG. **8** depicts an amplification plot of an exemplary mix 2 of a kit including OXA gene targets.

(9) FIG. **9** depicts an amplification plot of an exemplary internal control mix of a kit including SHV-TEM gene targets.

DETAILED DESCRIPTION

(10) The explanations and illustrations presented herein are intended to acquaint others skilled in

the art with the teachings, its principles, and its practical application. Those skilled in the art may adapt and apply the teachings in its numerous forms, as may be best suited to the requirements of a particular use. Accordingly, the specific embodiments of the present teachings as set forth are not intended as being exhaustive or limiting of the teachings. The scope of the teachings should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled. The disclosures of all articles and references, including patent applications and publications, are incorporated by reference for all purposes. Other combinations are also possible as will be gleaned from the following claims, which are also hereby incorporated by reference into this written description.

(11) Bacterial resistance to antibiotics poses a global threat to public health and in recent years has shown an increase in mortality rates and the potential to spread through the population. Of these resistance mechanisms, β-Lactamases are enzymes that cleave β-Lactam rings rendering the β-Lactam family of antibiotics ineffective for treatment of clinically-important Gram-negative bacterial infections. Specifically, β-Lactamases confer resistance to penicillins, cephamycins, and, in some cases, carbapenems. β-Lactam-resistant Gram-negative organisms, producing multiple or plasmid-mediated β-lactamases, are difficult to identify phenotypically and necessitate more specific detection methods to identify clinically important β-lactamases. Genetic identification of these resistance mechanisms is critical for active surveillance and infection control. Because these antibiotics are often selected for the management and prevention of infectious disease, the presence and characteristics of specific β-Lactamases play a critical role in selecting the appropriate antibiotic therapy.

(12) AmpC β-lactamases are clinically important cephalosporinases that are resistant to most β-lactam antibiotics. AmpC enzymes are chromosomally encoded in many bacterial species and can be inducible and overexpressed as a consequence of mutation. Overexpression can lead to resistance to most β-lactam antibiotics. The occurrence of transmissible plasmids with acquired genes for AmpC β-lactamases often result in increased β-lactamase production, compared to chromosomally-expressed ampC genes. Additionally, plasmid-mediated AmpC β-lactamases can appear in organisms lacking or having low-level expression of a chromosomal ampC gene. Resistance due to plasmid-mediated AmpC enzymes can be broad in spectrum and often hard to detect. As such, it is clinically useful to detect and discriminate between plasmid-mediated and chromosomally expressed AmpC β-lactamases.

(13) The present teachings relate to assays and methods for detecting Gram-negative bacteria resistant to beta-lactam antibiotics from a biological sample. β-lactam antibiotics are all antibiotic agents that contain a β-lactam ring in their molecular structures. β-lactam antibiotics include penicillins, cephalosporins, carbapenems and monobactams. Antibiotic resistant organisms may produce one or more enzymes known as β-lactamases that provide resistance to β-lactam antibiotics. β-lactamases may confer resistance by the bacteria to antibiotics, which is plasmid-mediated and/or chromosomally expressed making detection difficult.

(14) β-lactamases may be classified based on molecular structure. The four major classes include A to D. Class A, C and D β-lactamases are serine based. Class B β-lactamases, also known as metallo-beta-lactamases, are zinc based.

(15) Extended spectrum β-lactamases (ESBLs) are enzymes that confer bacterial resistance to certain categories of antibiotics, such as third-generation cephalosporins and monobactams. The presence of an ESBL-producing organism in a clinical infection can cause treatment failure if one of the above classes of drugs is used. Detection of ESBLs can be difficult because they have different levels of activity against various cephalosporins. Thus genetic identification of the exact enzyme can facilitate selection of the optimal antimicrobial agent, which is critical to determine the most effective treatment response.

(16) First-generation cephalosporins include cefalexin, cefaloridine, cefalotin, cefazolin, cefadroxil,

cefazedone, cefatrizine, cefapirin, cefradine, cefacetrile, cefrodaxine, ceftezole. Second-generation cephalosporins include cefoxitin, cefuroxime, cefamandole, cefaclor, cefotetan, cefonicide, cefotiam, loracarbef, cefmetazole, cefprozil, ceforanide. Third-generation cephalosporins include cefotaxime, ceftazidime, cefsulodine, ceftriaxone, cefmenoxime, latamoxef, ceftizoxime, cefixime, cefodizime, cefetamet, cefpiramide, cefoperazone, cefpodoxime, ceftibuten, cefdinir, cefditoren, ceftriaxone, cefoperazone, cefbuperazone. Fourth-generation cephalosporins include cefepime and cefpirome.

(17) β-lactamase producing bacteria may include Gram-negative bacteria such as those found in the following genera: *Pseudomonas, Escherichia, Salmonella, Shigella, Enterobacter, Klebsiella, Serratia, Proteus, Campylobacter, Haemophilus, Morganella, Vibrio, Yersinia, Acinetobacter, Branhamella, Neisseria, Burkholderia, Citrobacter, Hafnia, Edwardsiella, Aeromonas, Moraxella, Pasteurella, Providencia* and *Legionella.*

(18) Antibiotic resistance is intended to mean any type of mechanism which allows a microorganism to render a treatment partially or completely ineffective on the microorganism, guaranteeing its survival. β-lactam antibiotic resistance is intended to mean any type of β-lactamase-based mechanism which allows a microorganism to render a treatment partially or completely ineffective on the microorganism, guaranteeing its survival. For example, wherein the mechanism is related to the expression of an enzyme belonging to the β-lactamase group including extended-spectrum β-lactamase or of an enzyme belonging to the group of class C cephalosporinases.

(19) Biological sample is intended to mean a clinical sample, derived from a specimen of biological fluid, or a food sample, derived from any type of food or drink, or from an agricultural source, such as animals, soil, water, or air, or from a surface such as with a biofilm. This sample may thus be liquid or solid. For example the biological sample may be a clinical sample of blood, plasma, urine or feces, or of rectal, nose, throat, skin, wound or cerebrospinal fluid specimens.

(20) The present teachings relate to assays and methods for detecting resistance to beta-lactam antibiotics. The present teachings may detect β-lactamase gene targets which are chromosomally encoded and/or plasmid mediated. The present teachings provide for the detection of family specific gene targets relating to β-lactamase genes including AmpC β-lactamases. The β-lactamase genes detected with the present teachings may include those classified into molecular groups A through D. The β-lactamase genes detected with the present teachings may include those classified into functional groups 1 through 3.

(21) The present teachings relate to assays and methods for detecting resistance of one or more gene beta lactamase gene families including like genes. A like gene may be a beta-lactamase that has one or more of the following: similar amino acid sequence, similar function and similar antibiotic susceptibility profiles. A like gene may be considered as like the target gene detected with the present teachings. For example, OXA-48-like enzymes may include: OXA-48, OXA-48b, OXA-162, OXA-163, OXA-181, OXA-199, OXA-204, OXA-232, OXA-244, OXA-245 and OXA-24.

(22) The present teachings provide one or more primers and/or probes for the identification of one or more β-lactamase genes selected from the group consisting of: CMY, CTX-M, OXA, IMP, VIM, DHA, KPC, MOX, ACC, FOX, EBC, NDM, TEM, and SHV. The present teachings provide one or more primers and/or probes for the identification of β-lactamase genes selected from the group consisting of one or more of the following: MOX-like, FOX-like, ACC-like, EBC-like, CMY-2-like, DHA-like, CTX-M-14-like, CTX-M-15-like, VIM-like, NDM-like, IMP-like, KPC-like, and OXA-48-like, OXA-51-like, OXA-143-like, OXA-58-like, OXA-23-like, OXA-24/40-like, TEM-like, and SHV-like. The present teachings provide one or more primers and/or probes for the identification of a non-beta lactamase gene family which confers antibiotic resistance. For example, one or more primers and/or probes for the identification of MCR gene variants. The primers and/or probes of the present teachings may be included in one or more kits. The one or more kits may be

used for identification with any of the following: polymerase chain reaction, microarray, NGS-based target enrichment, and/or mass spectrometric characterization.

(23) Exemplary sequences for primers and probes for of the present teachings are depicted in Table 1. [SEQ. ID NOS 67-260] Primers and/or probes may be degenerate at any nucleotide position. Primers and/or probes may not be degenerate at any nucleotide position. Any suitable fluorophore and/or quencher and nucleic acid sequence combination may be used. For example, a probe may be labeled with a fluorescent tag at one end and a fluorescent quencher at the other end. For example, a probe may be labeled with a fluorescent tag at one end and a fluorescent quencher at the other end. For example, two fluorescent quenchers may be included at one end or within the probe sequence. It is contemplated that the probe sequences of the present teachings may be labeled with any suitable fluorophore and quencher combinations. For example, any fluorophore of the present teachings may be attached to any probe DNA sequence of the present teachings.

(24) TABLE-US-00001 TABLE 1 Primer/Probe Sequence SEQ ID NO. 67 TGGCCAGAACTGACAGGCAAA SEQ ID NO. 68 TTTCTCCTGAACGTGGCTGGC SEQ ID NO. 69 56-FAM/ACGCTAACT/ZEN/CCAGCATTGGTCTGT/3IABkFQ/ SEQ ID NO. 70 CCGTCACGCTGTTGTTAGG SEQ ID NO. 71 GCTGTGTTAATCAATGCCACAC SEQ ID NO. 72 5HEX/AACTTGCCG/ZEN/AATTAGAGCRGCAGT/3IABkFQ SEQ ID NO. 73 CGTTTCGTCTGGATCGCAC SEQ ID NO. 74 GCTGGGTAAAATAGGTCACC SEQ ID NO. 75 5TEX615/TATCATTGGTGGTGCCGTAGTCGC/3IAbRQSp SEQ ID NO. 76 GAGAGGATGAYCAGCCACAC SEQ ID NO. 77 CGCCCATTGTSCAATATTCC SEQ ID NO. 78 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp SEQ ID NO. 79 AATCACAGGGCGTAGTTGTG SEQ ID NO. 80 ACCCACCAGCCAATCTTAGG SEQ ID NO. 81 56-FAM/TAGCTTGAT/ZEN/CGCCCTCGATTTGGG/3IABkFQ/ SEQ ID NO. 82 GCGGAGTTAACTATTGGCTAG SEQ ID NO. 83 GGCCAAGCTTCTATATTTGCG SEQ ID NO. 84 5HEX/TTRTTYGGT/ZEN/GGTTGYTTTRTTAA/3IABkFQ SEQ ID NO. 85 GCGGAGTTARYTATTGGCTAG SEQ ID NO. 86 GGCCAAGCYTCTAWATTTGCG SEQ ID NO. 87 /5HEX/CCGGACGGT/ZEN/CTTGGTAATTTGGGT/3IABkFQ/ SEQ ID NO. 88 /5HEX/CCGTACGGT/ZEN/TTAGGCAATTTGGGT/3IABkFQ/ SEQ ID NO. 89 GGCGGCGTTGATGTCCTTCG SEQ ID NO. 90 CCATTCAGCCAGATCGGCATC SEQ ID NO. 91 5TEX615/AGCTCTTCTATCCTGGTGCTGCG/3IAbRQSp SEQ ID NO. 92 AACTTTCACAGGTGTGCTGGGT SEQ ID NO. 93 CCGTACGCATACTGGCTTTGC SEQ ID NO. 94 56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IABkFQ/ SEQ ID NO. 95 GTATCGCCGTCTAGTTCTGC SEQ ID NO. 96 CCTTGAATGAGCTGCACAGTGG SEQ ID NO. 97 5HEX/TCGTCGCGG/ZEN/AACCATTCGCTAAA/3IABkFQ/ SEQ ID NO. 98 GTTTGATCGTCAGGGATGGC SEQ ID NO. 99 GGCGAAAGTCAGGCTGTG SEQ ID NO. 100 5TEX615/CATCAGGACAAGATGGGCGGTATG/3IAbRQSp SEQ ID NO. 101 GCTGCTCAAGGAGCACAGGAT SEQ ID NO. 102 CACATTGACATAGGTGTGGTGC SEQ ID NO. 103 56-FAM/AGGATGGCA/ZEN/AGGCCCACTATTTCA/3IABkFQ SEQ ID NO. 104 AACAGCCTCAGCAGCCGGTTA SEQ ID NO. 105 TTCGCCGCAATCATCCCTAGC SEQ ID NO. 106 5HEX/AGCCATTAC/ZEN/GTTCCAGAGTTGCGT/3IABkFQ SEQ ID NO. 107 GCCGAGGCTTACGGGATCAAG SEQ ID NO. 108 CAAAGCGCGTAACCGGATTGG SEQ ID NO. 109 5TEX615/TCTGCTGAAGTTTRYCGAGGCMAA/3IAbRQSp SEQ ID NO. 110 AACTTTCACAGGTGTGCTGGGT SEQ ID NO. 111 CCGTACGCATACTGGCTTTGC SEQ ID NO. 112 56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IABkFQ/ SEQ

ID NO. 113 CTGGGTTCTATAAGTAAAACCTTCACCGG SEQ ID NO. 114 CTTCCACTGCGGCTGCCAGTT SEQ ID NO. 115 5HEX/GATGCCATT/ZEN/GCYCGSGGTGAAAT/3IABkFQ SEQ ID NO. 116 CCGAAGCCTATGGCGTGAAATCC SEQ ID NO. 117 GCAATGCCCTGCTGGAGCG SEQ ID NO. 118 5TEX615/ATGTTGGCCTGAACCCAGCG/3IAbRQSp SEQ ID NO. 119 AGCACATACAGAATATGTCCCTGC SEQ ID NO. 120 ACCTGTTAACCAACCTACTTGAGGG SEQ ID NO. 121 /56-FAM/TTGCAAGACGGACTGGCTTAGACC/3BHQ_1/ SEQ ID NO. 122 CCTGATCGGATTGGAGAACC SEQ ID NO. 123 CTACCTCTTGAATAGGCGTAACC SEQ ID NO. 124 /5TEX615/ACGTCGCGCAAGTTCCTGATAGAC/3IAbRQSp/ SEQ ID NO. 125 TAGTGACTGCTAATCCAAATCACAG SEQ ID NO. 126 GCACGAGCAAGATCATTACCATAGC SEQ ID NO. 127 /5HEX/AGTTATCCAACAAGGCCAAACTCAACA/3BHQ_1/ SEQ ID NO. 128 AATCACAGGGCGTAGTTGTG SEQ ID NO. 129 ACCCACCAGCCAATCTTAGG SEQ ID NO. 130 /5HEX/TAGCTTGATCGCCCTCGATTTGGG/3BHQ_1/ SEQ ID NO. 131 GTGGGATGGAAAGCCACG SEQ ID NO. 132 CACTTGCGGGTCTACAGC SEQ ID NO. 133 /56-FAM/TTACTTTGGGCGAAGCCATGCAAG/3BHQ_1/ SEQ ID NO. 134 CACCTATGGTAATGCTCTTGC SEQ ID NO. 135 CTGGAACTGCTGACAATGCC SEQ ID NO. 136 /5TEX615/TGGGAGAAAGATATGACTTTAGGTGAGGCA/3IAbRQSp/ SEQ ID NO. 137 CCGTGTATGTTCAGCTAT SEQ ID NO. 138 CTTATCCATCACGCCTTT SEQ ID NO. 139 /5TEX615/TATGATGTCGATACCGCCAAATACCA/3IAbRQSp/ SEQ ID NO. 140 CTGTATGTCAGCGATCAT SEQ ID NO. 141 GATGCCAGTTTGCTTATCC SEQ ID NO. 142 /56FAM/AAGTCTGGG/ZEN/TGAGAACGGTGTCTAT/3IABkFQ SEQ ID NO. 143 CAGTCAGTATGCGAGTTTC SEQ ID NO. 144 AAAATTCGCCAAGCCATC, SEQ ID NO. 145 /5HEX/TGCATAAGC/ZEN/CAGTGCGTTTTTATAT/3IABkFQ SEQ ID NO. 146 AGATCAGTTGGGTGCACG SEQ ID NO. 147 TGCTTAATCAGTGAGGCACC SEQ ID NO. 148 /56-FAM/ATGAAGCCA/ZEN/TACCAAACGACGAGC/3IABkFQ/ SEQ ID NO. 149 CTGGAGCGAAAGATCCACTA SEQ ID NO. 150 ATCGTCCACCATCCACTG SEQ ID NO. 151 /5HEX/CCAGATCGG/ZEN/CGACAACGTCACC/3IABkFQ/ SEQ ID NO. 152 TGGCCAGAACTGACAGGCAAA SEQ ID NO. 153 TTTCTCCTGAACGTGGCTGGC SEQ ID NO. 154 56-FAM/ACGCTAACT/ZEN/CCAGCATTGGTCTGT/3IABkFQ/ SEQ ID NO. 155 CCGTCACGCTGTTGTTAGG SEQ ID NO. 156 GCTGTGTTAATCAATGCCACAC SEQ ID NO. 157 5HEX/AACTTGCCG/ZEN/AATTAGAGCRGCAGT/3IABkFQ SEQ ID NO. 158 CGTTTCGTCTGGATCGCAC SEQ ID NO. 159 GCTGGGTAAAATAGGTCACC SEQ ID NO. 160 5TEX615/TATCATTGGTGGTGCCGTAGTCGC/3IAbRQSp SEQ ID NO. 161 GAGAGGATGAYCAGCCACAC SEQ ID NO. 162 CGCCCATTGTSCAATATTCC SEQ ID NO. 163 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp SEQ ID NO. 164 AATCACAGGGCGTAGTTGTG SEQ ID NO. 165 ACCCACCAGCCAATCTTAGG SEQ ID NO. 166 56-FAM/TAGCTTGAT/ZEN/CGCCCTCGATTTGGG/13IABkFQ/ SEQ ID NO. 167 GCGGAGTTAACTATTGGCTAG SEQ ID NO. 168 GGCCAAGCTTCTATATTTGCG SEQ ID NO. 169 5HEX/TTRTTYGGT/ZEN/GGTTGYTTTRTTAA/3IABkFQ SEQ ID NO. 170 GCGGAGTTARYTATTGGCTAG SEQ ID NO. 171 GGCCAAGCYTCTAWATTTGCG SEQ ID NO. 172 /5HEX/CCGGACGGT/ZEN/CTTGGTAATTTGGGT/3IABkFQ/ SEQ ID NO. 173 /5HEX/CCGTACGGT/ZEN/TTAGGCAATTTGGGT/3IABkFQ SEQ ID NO. 174 GGCGGCGTTGATGTCCTTCG SEQ ID NO. 175 CCATTCAGCCAGATCGGCATC

SEQ ID NO. 176 5TEX615/AGCTCTTCTATCCTGGTGCTGCG/3IAbRQSp SEQ ID NO. 177 GAGAGGATGAYCAGCCACAC SEQ ID NO. 178 CGCCCATTGTSCAATATTCC SEQ ID NO. 179 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp SEQ ID NO. 180 AACTTTCACAGGTGTGCTGGGT SEQ ID NO. 181 CCGTACGCATACTGGCTTTGC SEQ ID NO. 182 56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IABkFQ/ SEQ ID NO. 183 GTATCGCCGTCTAGTTCTGC SEQ ID NO. 184 CCTTGAATGAGCTGCACAGTGG SEQ ID NO. 185 5HEX/TCGTCGCGG/ZEN/AACCATTCGCTAAA/3IABkFQ/ SEQ ID NO. 186 GTTTGATCGTCAGGGATGGC SEQ ID NO. 187 GGCGAAAGTCAGGCTGTG SEQ ID NO. 188 5TEX615/CATCAGGACAAGATGGGCGGTATG/3IAbRQSp SEQ ID NO. 189 GAGAGGATGAYCAGCCACAC SEQ ID NO. 190 CGCCCATTGTSCAATATTCC SEQ ID NO. 191 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp SEQ ID NO. 192 GCTGCTCAAGGAGCACAGGAT SEQ ID NO. 193 CACATTGACATAGGTGTGGTGC SEQ ID NO. 194 56-FAM/AGGATGGCA/ZEN/AGGCCCACTATTTCA/3IABkFQ SEQ ID NO. 195 AACAGCCTCAGCAGCCGGTTA SEQ ID NO. 196 TTCGCCGCAATCATCCCTAGC SEQ ID NO. 197 5H EX/AGCCATTAC/ZEN/GTTCCAGAGTTGCGT/3IABkFQ SEQ ID NO. 198 GCCGAGGCTTACGGGATCAAG SEQ ID NO. 199 CAAAGCGCGTAACCGGATTGG SEQ ID NO. 200 5TEX615/TCTGCTGAAGTTTRYCGAGGCMAA/3IAbRQSp SEQ ID NO. 201 GAGAGGATGAYCAGCCACAC SEQ ID NO. 202 CGCCCATTGTSCAATATTCC SEQ ID NO. 203 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp SEQ ID NO. 204 AACTTTCACAGGTGTGCTGGGT SEQ ID NO. 205 CCGTACGCATACTGGCTTTGC SEQ ID NO. 206 56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IABkFQ SEQ ID NO. 207 CTGGGTTCTATAAGTAAAACCTTCACCGG SEQ ID NO. 208 CTTCCACTGCGGCTGCCAGTT SEQ ID NO. 209 5HEX/GATGCCATT/ZEN/GCYCGSGGTGAAAT/3IABkFQ SEQ ID NO. 210 CCGAAGCCTATGGCGTGAAATCC SEQ ID NO. 211 GCAATGCCCTGCTGGAGCG SEQ ID NO. 212 5TEX615/ATGTTGGCCTGAACCCAGCG/3IAbRQSp SEQ ID NO. 213 GAGAGGATGAYCAGCCACAC SEQ ID NO. 214 CGCCCATTGTSCAATATTCC SEQ ID NO. 215 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp SEQ ID NO. 216 AGCACATACAGAATATGTCCCTGC SEQ ID NO. 217 ACCTGTTAACCAACCTACTTGAGGG SEQ ID NO. 218 /56-FAM/TTGCAAGACGGACTGGCTTAGACC/3BHQ_1/ SEQ ID NO. 219 CCTGATCGGATTGGAGAACC SEQ ID NO. 220 CTACCTCTTGAATAGGCGTAACC SEQ ID NO. 221 /5TEX615/ACGTCGCGCAAGTTCCTGATAGAC/3IAbRQSp/ SEQ ID NO. 222 TAGTGACTGCTAATCCAAATCACAG SEQ ID NO. 223 GCACGAGCAAGATCATTACCATAGC SEQ ID NO. 224 /5HEX/AGTTATCCAACAAGGCCAAACTCAACA/3BHQ_1/ SEQ ID NO. 225 GAGAGGATGAYCAGCCACAC SEQ ID NO. 226 CGCCCATTGTSCAATATTCC SEQ ID NO. 227 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp SEQ ID NO. 228 AATCACAGGGCGTAGTTGTG SEQ ID NO. 229 ACCCACCAGCCAATCTTAGG SEQ ID NO. 230 /5HEX/TAGCTTGATCGCCCTCGATTTGGG/3BHQ_1/ SEQ ID NO. 231 GTGGGATGGAAAGCCACG SEQ ID NO. 232 CACTTGCGGGTCTACAGC SEQ ID NO. 233 /56-FAM/TTACTTTGGGCGAAGCCATGCAAG/3BHQ_1/ SEQ ID NO. 234 CACCTATGGTAATGCTCTTGC, SEQ ID NO. 235 CTGGAACTGCTGACAATGCC SEQ ID NO. 236 /5TEX615/TGGGAGAAAGATATGACTTTAGGTGAGGCA/3IAbRQSp/ SEQ ID NO. 237

GAGAGGATGAYCAGCCACAC SEQ ID NO. 238 CGCCCATTGTSCAATATTCC SEQ ID NO. 239 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp SEQ ID NO. 240 AGATCAGTTGGGTGCACG SEQ ID NO. 241 TGCTTAATCAGTGAGGCACC SEQ ID NO. 242 /56-FAM/ATGAAGCCA/ZEN/TACCAAACGACGAGC/3IABkFQ/ SEQ ID NO. 243 CTGGAGCGAAAGATCCACTA SEQ ID NO. 244 ATCGTCCACCATCCACTG SEQ ID NO. 245 /5HEX/CCAGATCGG/ZEN/CGACAACGTCACC/3IABkFQ/ SEQ ID NO. 246 GAGAGGATGAYCAGCCACAC SEQ ID NO. 247 CGCCCATTGTSCAATATTCC SEQ ID NO. 248 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp SEQ ID NO. 249 CCGTGTATGTTCAGCTAT SEQ ID NO. 250 CTTATCCATCACGCCTTT SEQ ID NO. 251 /5TEX615/TATGATGTCGATACCGCCAAATACCA/3IAbRQSp/ SEQ ID NO. 252 CTGTATGTCAGCGATCAT SEQ ID NO. 253 GATGCCAGTTTGCTTATCC SEQ ID NO. 254 /56FAM/AAGTCTGGG/ZEN/TGAGAACGGTGTCTAT/3IABkFQ/ SEQ ID NO. 255 CAGTCAGTATGCGAGTTTC SEQ ID NO. 256 AAAATTCGCCAAGCCATC SEQ ID NO. 257 /5HEX/TGCATAAGC/ZEN/CAGTGCGTTTTTATAT/3IABkFQ/ SEQ ID NO. 258 GAGAGGATGAYCAGCCACAC SEQ ID NO. 259 CGCCCATTGTSCAATATTCC SEQ ID NO. 260 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp

(25) The present teachings provide a molecular assay. The present teachings may provide a qualitative (i.e. end point) molecular assay for the detection of family-specific KPC, ESBL, MBL, and ampC gene targets. The present teachings may provide a qualitative (i.e. end point) molecular assay for the detection of family-specific plasmid-mediated ampC β-lactamase genes. The present teachings may provide a qualitative (i.e. end point) molecular assay for the detection of OXA gene targets. Fluorescently-labeled DNA probes may be used for detection. The assay of the present teachings may provide for differentiation between a plasmid-mediated ampC β-lactamase gene from a chromosomal ampC β-lactamase gene; provided the two genes are not from the same chromosomal origin. The assay may involve extraction of DNA from bacterial cells. The assay may include subsequent PCR amplification. The assay may include gel-based detection.

(26) In contrast, to traditional phenotypic methods which require 24-48 hours for data, the present teachings may provide for data generation in just hours or one hour. The total time required for DNA extraction, PCR set-up, amplification, and analysis may be around about 2 hours to about 3 hours. The sensitivity of the assay may be about 100%. The specificity of the assay may be about 100%. Therefore, the present teachings provide for fast and reliable detection. Implementation of such rapid assays have a positive impact for infection control and patient care.

(27) The present teachings allow for the detection of multiple β-lactamase gene families. The β-lactamases may include all major β-lactamases including ampC types. For example, the present teachings may allow for identification of up to six to nine β-lactamase gene families. The β-lactamase gene families may include CMY, CTX-Ms, DHA, IMP, KPC, NDM, OXA and VIM. The AmpC β-lactamases gene families may include MOX, ACC, FOX, DHA, CMY and EBC.

(28) The present teachings provide for a kit which allows for identification of at least nine β-lactamase gene families. The gene families may include: IMP-1-like, NDM-like, OXA-48-like, CTX-M-14-like, CTX-M-15-like, CMY-2-like, DHA-like, VIM-like, and KPC-like. The kit may also include an endogenous internal control (IC) that targets a conserved region common in gram-negative bacteria to reduce false negatives due to PCR inhibition, DNA degradation, or poor extraction. It is contemplated that the endogenous internal control discriminates false negative samples from true negative samples due to but not limited to one or more of PCR inhibition, DNA degradation, and/or poor extraction. The kit may utilize sequence-specific primer pairs for the PCR amplification of each target group. The kit may utilize fluorescently-labeled, target-specific DNA probes for detection by real-time PCR.

(29) The kit may include one or more multiplex primer-probe mixes containing one or more

primers and one or more probes. The multiplex primer-probe mix may be a 10×PCR mix. In one example, the kit includes three multiplex primers-probes mix vials. The mix vials may provide for simultaneous real-time PCR amplification of all targets between three reaction tubes. PCR Mix 1 may amplify a first set of three gene families. For example, CMY-2, CTX-M-14, and CTX-M-15. PCR Mix 2 may amplify a second set of three gene families. For example, OXA-48, IMP, and VIM. PCR mix 3 may amplify a third set of gene families. For example, DHA, KPC, and NDM. The multiplex mix may also include an internal control (IC) in each mix. The kit may include three external DNA control vials or first control mix vial, a second control mix vial and a third control mix vial. The DNA control mix vial may contain synthetic DNA templates of the corresponding multiplex targets. The DNA control mixes may serve as a positive control for each multiplex reaction. The DNA control mix may contain stabilized bacteria with chromosomal or transmissible genetic elements in a sample matrix similar to a patient sample.

(30) The present teachings provide for a kit which allows for identification of at least six plasmid-mediated ampC gene families. The gene families may include: MOX-like, DHA-like, ACC-like, EBC-like, FOX-like, and CMY-2-like. The kit may also include an endogenous internal control (IC) that targets a conserved region common in gram-negative bacteria to reduce false negatives due to PCR inhibition, DNA degradation, or poor extraction. It is contemplated that the endogenous internal control discriminates false negative samples from true negative samples due to but not limited to one or more of PCR inhibition, DNA degradation, and/or poor extraction. The kit may utilize sequence-specific primer pairs for the PCR amplification of each family. The kit may utilize fluorescently-labeled, target-specific DNA probes for detection by real-time PCR.

(31) The kit may include one or more multiplex primer-probe mixes containing one or more primers and one or more probes. The multiplex primer-probe mix may be a 10×PCR mix. In one example, the kit includes two multiplex primers-probes mix vials. The mix vials may provide for simultaneous real-time PCR amplification of all targets between two reaction tubes. PCR Mix 1 may amplify a first set of three gene families. For example, MOX, ACC and FOX. PCR Mix 2 may amplify a second set of three gene families. For example, DHA, EBC and CMY-2. The multiplex mix may also include an internal control (IC) in each mix. The kit may include two external DNA control vials or first control mix vial and a second control mix vial. The DNA control mix vial may contain synthetic DNA templates of the corresponding multiplex targets. The DNA control mixes may serve as a positive control for each multiplex reaction.

(32) The present teachings provide for a kit which allows for identification of at least six OXA carbapenemase gene families. The gene families may include: OXA-23, OXA-24/40, OXA-48, OXA-51, OXA-58, and OXA-143. The gene families may include like gene families. The kit may also include an endogenous internal control (IC) that targets a conserved region common in gram-negative bacteria to reduce false negatives due to PCR inhibition, DNA degradation, or poor extraction. It is contemplated that the endogenous internal control discriminates false negative samples from true negative samples due to but not limited to one or more of PCR inhibition, DNA degradation, and/or poor extraction. The kit may utilize sequence-specific primer pairs for the PCR amplification of each family. The kit may utilize fluorescently-labeled, target-specific DNA probes for detection by real-time PCR.

(33) The kit may include one or more multiplex primer-probe mixes containing one or more primers and one or more probes. The multiplex primer-probe mix may be a 10×PCR mix. In one example, the kit includes two multiplex primers-probes mix vials. The mix vials may provide for simultaneous real-time PCR amplification of all targets between two reaction tubes. PCR Mix 1 may amplify a first set of three gene families. For example, OXA 143, OXA 23 and OXA 51. PCR Mix 2 may amplify a second set of three gene families. For example, OXA 24/40, OXA-48 and OXA-58. The multiplex mix may also include an internal control (IC) in each mix. The kit may include two external DNA control vials or first control mix vial and a second control mix vial. The DNA control mix vial may contain synthetic DNA templates of the corresponding multiplex

targets. The DNA control mixes may serve as a positive control for each multiplex reaction.
(34) In addition, the present teachings contemplate that the kit or kits of the present teachings may provide for the detection of a non-beta lactamase gene family. The kit or kits may provide for detection of plasmid-mediated mechanisms of antibiotic resistance for one more types/categories of antibiotics. For example, the kit may also provide for the detection of the MCR-1 gene which confers polymixin resistance. The kit or kits may include primer sequences, probe sequences, and a control sequence for detection of one or more non-beta lactamase gene family in addition to beta-lactamase genes. For example, a kit may provide for the detection of ampC genes families and a MCR-1 gene family.
(35) Furthermore, the present teachings allow for the expansion of the detection of other β-lactamase gene families including TEM and SHV. The gene families may include like gene families. The kit may also include an endogenous internal control (IC) that targets a conserved region common in gram-negative bacteria to reduce false negatives due to PCR inhibition, DNA degradation, or poor extraction. It is contemplated that the endogenous internal control discriminates false negative samples from true negative samples due to but not limited to one or more of PCR inhibition, DNA degradation, and/or poor extraction. The kit may utilize sequence-specific primer pairs for the PCR amplification of each family. The kit may utilize fluorescently-labeled, target-specific DNA probes for detection by real-time PCR.
(36) The kit or kits of the present teachings may include synthetic DNA oligonucleotide primers, target-specific DNA probes and DNA controls for the specified gene targets suspended in TE buffer, pH 8.0. The contents of the kit may be enclosed in vials. For example, the one or more 10×PCR mixes may be comprised of 275 μL. For example, the one or more control mixes may be comprised of 14 μL. For example, the contents of the kit may be sufficient for about 100 reactions total and about 12 reactions of the control DNA mix.
(37) Detection of each target is based on the optical fluorescence of the fluorophore conjugated to each target-specific DNA probe. Any suitable fluorophore and nucleic acid sequence combination may be used. For example, the fluorophores may be selected from the group consisting of: FAM (6-Carboxyfluorescein), HEX (Hexachlorofluorescein), TEX615 and TYE665.
(38) The present teachings provide assays for the detection of β-lactamase gene families from a biological sample. The assays may be included in a kit or kits. The kit may provide for the detection of β-lactamase by various molecular biology technologies and platforms. The kit may include one or more primers and/or probes for the identification by polymerase chain reaction or microarray of one or more β-lactamase genes selected from the group consisting of: CMY, CTX-M, OXA, IMP, VIM, DHA, KPC, MOX, ACC, FOX, EBC, NDM, TEM, and SHV.
(39) The kit may include one or more primers and/or probes for the identification by polymerase chain reaction or microarray of a non-beta lactamase gene family which confers antibiotic resistance. The kit may include one or more primers and/or probes for the identification by polymerase chain reaction or microarray of one or more MCR genes. The kit may include one or more primers and/or probes for the identification by polymerase chain reaction or microarray of a MCR-1 gene.
(40) The kit may provide for detection of specified targets from crude biological samples such as blood, urine, plasma, feces, sputum, etc. The kit may provide for detection of specified targets directly from or extracted directly from crude biological samples including but not limited to blood, blood cultures, urine, plasma, feces, fecal swabs, peri-rectal/peri-anal swabs, sputum, and bacterial cultures.
(41) The kit may be used for detection of specified targets from purified nucleic acid samples. The kit may be used for any nucleic acid amplification methodology. The kit may be used with conventional polymerase chain reaction. The kit may be used with real-time polymerase chain reaction. The kit may be used with digital droplet polymerase chain reaction. The kit may be used with detection by microarray technology. The kit may be used with fluorescence and/or infra-red

probe-based detection chemistries. The kit may be used with intercalating dye-based detection chemistries. The kit may be used for detection of nucleic acid polymerase chain reaction amplicons ranging from 25 base pairs to 2000 base pairs.

(42) The kit may include various reagents. The various reagents may be contained in various vials. The kit may include a primer set or primer sets. The primer set or primer sets may be labeled or unlabeled with a tracking dye or fluorophore. The kit may include probes. The kit may include a primer-probe mix. The kit may include controls. The kit may include magnesium chloride. The kit may include dNTPs. The kit may include DNA polymerase. The kit may include a tracking dye. The kit may include a composition containing a tracking dye. The kit may include a written protocol. The kit may include a customized master mix in a single tube, two tubes, three tubes, or four tubes containing all chemicals and enzymes necessary to run the PCR assay described herein. The kit may include freeze-dried or lyophilized reagents in a single assay tube or multiple assay tubes. The kit may provide for detection of nucleic acid and the kit reagents may be provided in any liquid form, pooled reaction mix, or lyophilized, freeze dried, or cryo-preserved format.

(43) The kit may include a primer set. The primer set may include at least one primer pair. A primer pair may include a forward primer and a reverse primer. The primer set may include one pair of primers. The primer set may include more than one pair of primers. The primer set may include two pairs of primers. The primer set may include three pairs of primers. The primer set may include one to six pairs of primers. The primer set may include one to ten pairs of primers. The primer set may include up to 30 pairs of primers. The primer set may include up to 50 pairs of primers. The primer set may include up to 100 pairs of primers.

(44) The kit may include a primer-probe mix. The primer-probe mix may include a primer set. The primer-probe mix may include one or more probes. Each pair of primers of the primer set may include a probe or set of probes. The primer-probe mix may include a pair of internal control primers. The pair of internal control primers may include a forward primer and a reverse primer. The primer-probe mix may include an internal control probe.

(45) For example, a primer-probe mix may include one or more pairs of primers, one associated probe per primer pair and internal controls including a pair of primers and a probe. Preferably, the primer-probe mix is a multiplex mix including more than one pair of primers, a probe for each primer pair and internal controls. The multiplex mix may be used for the identification of more than one β-lactamase gene family. Each primer pair and probe may detect a different β-lactamase gene family. For example, three primer pairs and their associated three probes may be used for detection of three different β-lactamase gene families.

(46) The DNA concentration range of each primer set in a PCR may be about 1 nM to about 10 μM (10,000 nM). One or more primers may be labeled with a florescent marker as a probe. The DNA concentration of each probe in a PCR may be about 1 nM to about 10,000 nM. The DNA concentration of each probe in a PCR may be about 10 to about 500 nM.

(47) The kit may include at least one control. The kit may include one, two, three or four controls. The kit may include one or more negative controls. The negative control may include nucleic acid known to express a resistance gene other than the target gene of interest. The kit may include one or more positive controls. The one or more positive controls may be internal controls. The positive control may include nucleic acid known to express or contain the resistance gene. The kit may include an endogenous internal control to reduce false negatives due to PCR inhibition, DNA degradation, and/or poor extraction. It is contemplated that the endogenous internal control discriminates false negative samples from true negative samples due to but not limited to one or more of PCR inhibition, DNA degradation, and/or poor extraction. The endogenous internal control may target a conserved nucleotide sequence or sequences common to the Gram-negative bacteria genome. For example, the internal control may detect the 16S rRNA and/or 23S rRNA gene(s). The internal control may detect the 16S and/or 23S rRNA gene for *E. coli, Pseudomonas, Acinetobacter, Klebsiella* and *Salmonella.*

(48) The kit may include control vector in the control vial. One or more μls of the vector control may be added to a 25 μl reaction to get the working concentration. The DNA concentrations for each control vector may be equivalent to 0.1 copy to 2000 copies or 0.0000243 pg/uL to 0.0455 pg/uL. The DNA concentrations for each control vector may be equivalent to 10 copies to 5000 copies or 0.001 pg/uL to 0.5 pg/uL. Control vector concentrations may be as high as 1×10(9) copies and any dilution thereof.

(49) The assays of the present teachings may include the use of magnesium chloride. The kit may include magnesium chloride. The assay may be utilized with a concentration of about 2 mM to about 7 mM MgCl.sub.2. Preferably, the concentration is about 3.0 mM to about 5.5 mM MgCl.sub.2. More preferably, the concentration is 5.0 mM MgCl.sub.2 for an assay for the detection of β-lactamase genes. More preferably the concentration is 5.0 mM MgCl.sub.2 for an assay for the detection of ampC β-lactamase genes. More, preferably, the concentration is 5 mM MgCl.sub.2 for an assay for the detection of OXA genes.

(50) The assays of the present teachings may include the use of DNA polymerase. The kit may include DNA polymerase. The assay may be utilized with a concentration of about 0.25 U/25 ul reaction to about 3 U/25 ul reaction of DNA polymerase. Preferably, the concentration is 1.25 U/25 μl reaction DNA polymerase for an assay for the detection of β-lactamase genes. Preferably the concentration is 1.25 U/25 μl DNA polymerase for an assay for the detection of β-lactamase ampC genes. For example, the present teachings may utilize the PhilisaFAST® DNA polymerase.

(51) The assays and methods of the present teachings may include a PCR cycling protocol. In one example, the cycling protocol comprises (1) 95° C. for 30 s; (2) 95° C. for 1 s; (3) 55° C. for 10 s; (4) 68° C. for 20 s; and repeating steps (2) to (4) for 40 cycles. In one example, the cycling protocol comprises (1) 95° C. for 30 s; (2) 95° C. for 6 s; (3) 66° C. for 10 s; and repeating steps (2) to (3) for 40 cycles. In one example, the cycling protocol includes a hot start of 98° C. for 30 s and 30 cycles of: 98° C. for 5 s, 60° C. for 10 s and 72° C. for 20 s. In one example, the cycling protocol includes using 98° C. for 30 s, followed by 30 cycles of 98° C. for 5 s, 60° C. for 10 s., and 72° C. for 25 s. In one example, the PCR protocols include a detection step where fluorescent signal is measured.

(52) The kit may include one or more of the following: primer, probe and control. A mix of one or more of the following: primer, probe and internal control, may be enclosed in one container. A mix of one or more of the following: primer, probe and internal control, may be enclosed in more than one container. The container may be a vial. In one example, the kit includes 3 DNA control vials and 3 10× primer/probe mix vials. Nine antibiotic resistance gene families and one internal control may be identified with the vials. In one example, the kit includes 2 DNA control vials and 2 10× primer/probe mix vials. Six antibiotic resistance gene families and one internal control may be identified with the vials.

(53) The present teachings allow for detection of the β-lactamase CMY-2 gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the CMY-2-like gene family. The biological sample may include Gram-negative bacteria such as *Escherichia coli, Proteus mirabilis, Klebsiella pneumoniae, Klebsiella oxytoca, Serratia marcescens, Citrobacter freundii* and other *Citrobacter* species. The CMY-2-like genes detected may include CMY-2, CMY-4, CMY-6, CMY-7, CMY-12, CMY-14, CMY-15, CMY-16, CMY-18, CMY-21, CMY-22, CMY-23, CMY-24, CMY-25, CMY-26, CMY-27, CMY-28, CMY-29, CMY-30, CMY-31, CMY-32, CMY-33, CMY-34, CMY-35, CMY-37, CMY-38, CMY-39, CMY-40, CMY-41, CMY-42, CMY-43, CMY-44, CMY-45, CMY-46, CMY-47, CMY-48, CMY-49, CMY-50, CMY-51, CMY-53, CMY-54, CMY-55, CMY-56, CMY-57, CMY-58, CMY-59, CMY-60, CMY-61, CMY-62, CMY-63, CMY-64, CMY-65, CMY-66, CMY-67, CMY-68, CMY-69, CMY-71, CMY-72, CMY-73, CMY-75, CMY-76, CMY-77, CMY-78, CMY-79, CMY-80, CMY-81, CMY-84, CMY-85, CMY-86, CMY-87, CMY-89, CMY-90, CMY-96, CMY-97, CMY-99, CMY-102, CMY-103, CMY-104,

CMY-105, CMY-107, CMY-108, CMY-110, CMY-111, CMY-112, CMY-113, CMY-114, CMY-115, CMY-116, CMY-117, CMY-118, CMY-119, CMY-121, CMY-122, CMY-124, CMY-125, CMY-126, CMY-127, CMY-128, CMY-129, CMY-130, CMY-131, CMY-132, CMY-133 and CMY-135.

(54) The present teachings allow for the detection of the β-lactamase CTX-M gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the CTX-M-14-like gene family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae, Enterobacter cloacae, Escherichia coli, Salmonella enterica, Proteus mirabilis* and *Shigella* species. The CTX-M-14-like genes detected may include CTX-M-9, CTX-M-13, CTX-M-14, CTX-M-16, CTX-M-17, CTX-M-19, CTX-M-21, CTX-M-24, CTX-M-27, CTX-M-38, CTX-M-51, CTX-M-64, CTX-M-65, CTX-M-67, CTX-M-82, CTX-M-83, CTX-M-84, CTX-M-85, CTX-M-86, CTX-M-90, CTX-M-93, CTX-M-98, CTX-M-99, CTX-M-102, CTX-M-104, CTX-M-105, CTX-M-110, CTX-M-111, CTX-M-112, CTX-M-113, CTX-M-121, CTX-M-122, CTX-M-123, CTX-M-125, CTX-M-129, CTX-M-130, CTX-M-132, CTX-M-134, CTX-M-147, CTX-M-148 and CTX-M-159.

(55) The present teachings allow for the detection of the β-lactamase CTX-M gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the CTX-M-15-like gene family. The biological sample may include Gram-negative bacteria such as *Escherichia coli, Klebsiella pneumoniae, Citrobacter freundii, Shigella* species and *Proteus mirabilis*. The CTX-M-15-like genes detected may include CTX-M-1, CTX-M-3, CTX-M-10, CTX-M-15, CTX-M-22, CTX-M-28, CTX-M-29, CTX-M-30, CTX-M-32, CTX-M-37, CTX-M-55, CTX-M-64, CTX-M-71, CTX-M-103, CTX-M-117, CTX-M-123, CTX-M-132, CTX-M-136, CTX-M-138, CTX-M-142, CTX-M-144, CTX-M-155, CTX-M-156, CTX-M-157, CTX-M-158, CTX-M-163, CTX-M-164, CTX-M-166 and CTX-M-172.

(56) The present teachings allow for the detection of the β-lactamase DHA gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the DHA-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae, Morganella morganii, Escherichia coli, Enterobacter cloacae, Proteus mirabilis* and *Citrobacter koseri*. The DHA-like genes detected may include DHA-1, DHA-2, DHA-5, DHA-6, DHA-7, DHA-9, DHA-10, DHA-12, DHA-13, DHA-14, DHA-15, DHA-16, DHA-17, DHA-18, DHA-19, DHA-20, DHA-21 and DHA-22.

(57) The present teachings allow for the detection of the β-lactamase IMP gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the IMP-like family. The biological sample may include Gram-negative bacteria such as *Serratia marcescens, Escherichia coli* and *Pseudomonas aeruginosa*. The IMP-like genes detected may include IMP-1, IMP-2, IMP-3, IMP-4, IMP-5, IMP-6, IMP-7, IMP-8, IMP-9, IMP-10, IMP-13, IMP-14, IMP-15, IMP-16, IMP-18, IMP-19, IMP-20, IMP-22, IMP-24, IMP-25, IMP-26, IMP-27, IMP-28, IMP-30, IMP-32, IMP-33, IMP-34, IMP-37, IMP-38, IMP-40, IMP-42, IMP-45, IMP-48, IMP-49, IMP-51 and IMP-52.

(58) The present teachings allow for the detection of the β-lactamase KPC gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the KPC-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae, Escherichia coli, Enterobacter cloacae* and other *Enterobacter* species, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The KPC-like genes detected may include KPC-1, KPC-2, KPC-3, KPC-4, KPC-5, KPC-6 KPC-7, KPC-8, KPC-9, KPC-10, KPC-11, KPC-

13, KPC-14, KPC-15, KPC-16, KPC-17 KPC-18, KPC-19, KPC-21, KPC-22, KPC-47, KPC-56, KPC-63, KPC-272, KPC-484, KPC-629, KPC-727, and KPC-860.

(59) The present teachings allow for the detection of the β-lactamase NDM gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the NDM-like family. The biological sample may include Gram-negative bacteria such as *Escherichia coli, Acinetobacter baumannii, Enterobacter cloacae* and *Klebsiella pneumoniae*. The NDM-like genes detected may include NDM-1, NDM-2, NDM-3, NDM-4, NDM-5, NDM-6, NDM-7, NDM-8, NDM-9, NDM-10, NDM-11, NDM-12, NDM-13, NDM-15, NDM-16 and NDM-32.

(60) The present teachings allow for the detection of the β-lactamase OXA gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the OXA-48-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae, Enterobacter cloacae, Shewanella xiamenensis, Escherichia coli* and *Serratia marcescens*. The OXA-48-like genes detected may include OXA-48, OXA-162, OXA-163, OXA-181, OXA-199, OXA-204, OXA-232, OXA-244, OXA-245, OXA-247, OXA-370, OXA-405, OXA-416, OXA-438 and OXA-439.

(61) The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including one or more of the following: OXA-143-like, OXA-23-like, OXA-51-like, OXA-48-like, OXA-58-like and OXA24/40-like. The OXA-143-like genes detected may include the following: OXA-143, OXA-182, OXA-231, OXA-253, and OXA-255. The OXA-23-like genes detected may include the following: OXA-23, OXA-27, OXA-49, OXA-73, OXA-102, OXA-103, OXA-105, OXA-133, OXA-134, OXA-146, OXA-165, OXA-166, OXA-167, OXA-168, OXA-169, OXA-170, OXA-171, OXA-225 and OXA-239. The OXA-51-like genes detected may include the following: OXA-51, OXA-64, OXA-65, OXA-66, OXA-67, OXA-68, OXA-69, OXA-70, OXA-71, OXA-75, OXA-76, OXA-77, OXA-78, OXA-79, OXA-80, OXA-82, OXA-83, OXA-84, OXA-86, OXA-87, OXA-88, OXA-89, OXA-90, OXA-91, OXA-92, OXA-93, OXA-94 OXA-95, OXA-98, OXA-99, OXA-100, OXA-104, OXA-106, OXA-107, OXA-108, OXA-109, OXA-110, OXA-111, OXA-112, OXA-113, OXA-115, OXA-116, OXA-117, OXA-120, OXA-121, OXA-122, OXA-123, OXA-124, OXA-125, OXA-126, OXA-127, OXA-128, OXA-130, OXA-131, OXA-132, OXA-138, OXA-144, OXA-148, OXA-149, OXA-150, OXA-172, OXA-173, OXA-174, OXA-175, OXA-176, OXA-177, OXA-178, OXA-179, OXA-180, OXA-194, OXA-195, OXA-196, OXA-197, OXA-200, OXA-201, OXA-202, OXA-203, OXA-206, OXA-208, OXA-216, OXA-217, OXA-219, OXA-223, OXA-241, OXA-242, OXA-248, OXA-249, OXA-250 and OXA-254. The OXA-48-like genes detected may include the following: OXA-48, OXA-48b, OXA-162, OXA-163, OXA-181, OXA-199, OXA-204, OXA-232, OXA-244, OXA-245 and OXA-247. The OXA-58-like genes may include the following: OXA-58, OXA-96, OXA-97 and OXA-164. The OXA-40-like genes may include the following: OXA-40, OXA-25, OXA-26, OXA-72, OXA-139, OXA-160 and OXA-207.

(62) The present teachings allow for the detection of the β-lactamase VIM gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the VIM-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella oxytoca, Citrobacter freundii, Klebsiella pneumoniae, Pseudomonas aeruginosa, Escherichia coli* and *Enterobacter cloacae*. The VIM-like genes detected may include VIM-1, VIM-2, VIM-3, VIM-4, VIM-5, VIM-6, VIM-8, VIM-9, VIM-10, VIM-11, VIM-12, VIM-13, VIM-14, VIM-15, VIM-16, VIM-17, VIM-18, VIM-19, VIM-20, VIM-23, VIM-24, VIM-25, VIM-26, VIM-27, VIM-28, VIM-31, VIM-33, VIM-34, VIM-35, VIM-36, VIM-37, VIM-38, VIM-39,

VIM-40, VIM-41, VIM-42, VIM-43, VIM-44, VIM-45 and VIM-46.
(63) The present teachings allow for the detection of the AmpC β-lactamase MOX gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the MOX-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae, Aeromonas punctata/Aeromonas caviae* and other *Aeromonas* species and *Escherichia coli*. The MOX-like genes detected may include MOX-1, MOX-2, MOX-3, MOX-4, MOX-5, MOX-6, MOX-7, MOX-8, MOX-10, CMY-1, CMY-8, CMY-9, CMY-10, CMY-11 and CMY-19.
(64) The present teachings allow for the detection of the AmpC β-lactamase ACC gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the ACC-like family. The biological sample may include Gram-negative bacteria such as *Salmonella enterica, Escherichia coli, Hafnia alvei* and *Proteus mirabilis*. The ACC-like genes detected may include ACC-1, ACC-2, ACC-4, ACC-5 and ACC-6.
(65) The present teachings allow for the detection of the AmpC β-lactamase FOX gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the FOX-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae* and *Aeromonas punctata*. The FOX-like genes detected may include FOX-1, FOX-2, FOX-3, FOX-4, FOX-5, FOX-6, FOX-7, FOX-8, FOX-9, FOX-10 and FOX-12.
(66) The present teachings allow for the detection of the AmpC β-lactamase DHA gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the DHA-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae, Morganella morganii, Escherichia coli* and *Enterobacter cloacae*. The DHA-like genes detected may include DHA-1, DHA-2, DHA-5, DHA-6, DHA-7, DHA-9, DHA-10, DHA-12, DHA-13, DHA-14, DHA-15, DHA-16, DHA-17, DHA-18, DHA-19, DHA-20, DHA-21 and DHA-22.
(67) The present teachings allow for the detection of the AmpC β-lactamase CMY-2 gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the CMY-2-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae, Morganella morganii, Escherichia coli* and *Enterobacter cloacae*. The CMY-2-like genes detected include CMY-2, CMY-4, CMY-6, CMY-7, CMY-12, CMY-14, CMY-15, CMY-16, CMY-18, CMY-21, CMY-22, CMY-23, CMY-24, CMY-25, CMY-26, CMY-27, CMY-28, CMY-29, CMY-30, CMY-31, CMY-32, CMY-33, CMY-34, CMY-35, CMY-37, CMY-38, CMY-39, CMY-40, CMY-41, CMY-42, CMY-43, CMY-44, CMY-45, CMY-46 CMY-47, CMY-48, CMY-49, CMY-50, CMY-51, CMY-53, CMY-54, CMY-55, CMY-56, CMY-57, CMY-58, CMY-59, CMY-60, CMY-61, CMY-62, CMY-63, CMY-64, CMY-65, CMY-66, CMY-67, CMY-68, CMY-69, CMY-71, CMY-72, CMY-73, CMY-75, CMY-76, CMY-77, CMY-78, CMY-79, CMY-80, CMY-81, CMY-84, CMY-85 CMY-86, CMY-87, CMY-89, CMY-90, CMY-96, CMY-97, CMY-99, CMY-102, CMY-103, CMY-104 CMY-105, CMY-107, CMY-108, CMY-110, CMY-111, CMY-112, CMY-113, CMY-114, CMY-115 CMY-116, CMY-117, CMY-118, CMY-119, CMY-121, CMY-122, CMY-124, CMY-125, CMY-126, CMY-127, CMY-128, CMY-129, CMY-130, CMY-131 CMY-132, CMY-133 and CMY-135.
(68) The present teachings allow for the detection of the AmpC β-lactamase EBC gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the EBC-like family such as ACT and MIR. The biological sample may include Gram-

negative bacteria such as *Enterobacter cloacae, Klebsiella pneumoniae, Enterobacter asburiae, Enterobacter kobei*, and other *Enterobacter* species. The EBC-like genes detected may include ACT-1, ACT-2, ACT-5, ACT-8, ACT-13, ACT-14, ACT-15, ACT-16, ACT-17, ACT-18, ACT-20, ACT-21, ACT-23, ACT-24, ACT-25, ACT-27, ACT-29, ACT-30, ACT-31, ACT-32, ACT-33, ACT-34, ACT-35, ACT-36, ACT-37, ACT-38, MIR-1, MIR-2, MIR-3, MIR-4, MIR-6, MIR-7, MIR-8, MIR-9, MIR-10, MIR-11, MIR-12, MIR-13, MIR-14, MIR-15, MIR-16, MIR-17 and MIR-18.

(69) The present teachings may allow for the detection of the β-lactamase TEM gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the TEM-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae, Enterobacter cloacae, Shewanella xiamenensis, Escherichia coli* and *Serratia marcescens*. The TEM-like genes detected may include TEM-1, TEM-2, TEM-3, TEM-15, TEM-20, TEM-32, TEM-40, TEM-52, TEM-88, TEM-91, TEM-97, TEM-98, TEM-106, TEM-107, TEM-112, TEM-120, TEM-126, TEM-135, TEM-141, TEM-150, TEM-153, TEM-163, TEM-168, TEM-170, TEM-171, TEM-206, TEM-214, and TEM-220.

(70) The present teachings may allow for the detection of the β-lactamase SHV gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of β-lactamase genes including the SHV-like family. The biological sample may include Gram-negative bacteria such as *Klebsiella pneumoniae, Enterobacter cloacae, Shewanella xiamenensis, Escherichia coli* and *Serratia marcescens*. The SHV-like genes detected may include SHV-1, SHV-2, SHV-3, SHV-5, SHV-7, SHV-8, SHV-9, SHV-11, SHV-12, SHV-13, SHV-14, SHV-15, SHV-16, SHV-18, SHV-24, SHV-25, SHV-26, SHV-27, SHV-28, SHV-29, SHV-30, SHV-31, SHV-32, SHV-33, SHV-34, SHV-35, SHV-36, SHV-37, SHV-38, SHV-40, SHV-41, SHV-42, SHV-43, SHV-44, SHV-45, SHV-46, SHV-48, SHV-49, SHV-50, SHV-51, SHV-52, SHV-53, SHV-55, SHV-56, SHV-57, SHV-59, SHV-60, SHV-61, SHV-62, SHV-63, SHV-64, SHV-65, SHV-66, SHV-67, SHV-69, SHV-70, SHV-71, SHV-72, SHV-73, SHV-74, SHV-75, SHV-76, SHV-77, SHV-78, SHV-79, SHV-80, SHV-81, SHV-82, SHV-85, SHV-86, SHV-89, SHV-92, SHV-93, SHV-94, SHV-95, SHV-96, SHV-97, SHV-98, SHV-99, SHV-100, SHV-101, SHV-102, SHV-103, SHV-104, SHV-105, SHV-106, SHV-107, SHV-109, SHV-110, SHV-111, SHV-119, SHV-120, SHV-121, SHV-122, SHV-123, SHV-124, SHV-125, SHV-126, SHV-127, SHV-128, SHV-129, SHV-132, SHV-133, SHV-134, SHV-135, SHV-136, SHV-137, SHV-140, SHV-141, SHV-142, SHV-143, SHV-144, SHV-145, SHV-146, SHV-147, SHV-148, SHV-149, SHV-150, SHV-151, SHV-152, SHV-153, SHV-154, SHV-155, SHV-156, SHV-157, SHV-158, SHV-159, SHV-160, SHV-161, SHV-162, SHV-163, SHV-164, SHV-165, SHV-168, SHV-172, SHV-173, SHV-178, SHV-179, SHV-180, SHV-182, SHV-183, SHV-185, SHV-186, SHV-187, SHV-188, SHV-189, SHV-190, SHV-191, SHV-193, SHV-194, SHV-195, SHV-196, and SHV-197.

(71) The present teachings may allow for the detection of the MCR gene family from a biological sample. The present teachings provide for a kit including one or more primers and/or probes for the identification by multiplex real-time polymerase chain reaction of MCR genes including the MCR-like family. The MCR-like genes detected may include MCR-1, MCR-1.2, MCR-1.3, MCR-1.4, MCR-1.5, MCR-1.6, MCR-1.7, MCR-1.8, MCR-1.9 and MCR-2.

(72) The kit of the present teachings may include a mix of at least one primer and/or at least one probe. Primers and/or probes may be degenerate at any nucleotide position. Primers and/or probes may not be degenerate at any nucleotide position. A hydrolysis and/or hybridization probe may be designed for the detection of a specific nucleic acid sequence. Multiple probes may be labeled with a different colored fluorophore. The probe may be labeled with a fluorescent tag at one end and a fluorescent quencher at the other end. Two fluorescent quenchers may be included at one end or within the probe sequence. For example, the fluorophores may be selected from the group consisting of fluorescein, hexachlorofluorescein, TEX 615, and TYE™ 665. The fluorophores may

excite between 450 nm and 763 nm and emit between 500 nm and 800 nm. For example, the quenchers may be selected from the group consisting of Iowa Black® quenchers and Black Hole Quenchers®. Peak absorbance of each quencher may be at 531 nm, 534 nm, 578 nm, or 656 nm.
(73) Multiple hydrolysis and/or hybridization probes can be added to the same nucleic acid amplification reaction. The selection of the fluorescent labels may depend on the type of hydrolysis and/or hybridization probe used, the number of targets to be detected and the type of thermal cycler used. Preferable combinations of fluorophores and quenchers for multiplex reactions require appropriate excitation wavelengths and little to no overlap in their emission spectra as well as reduction of background fluorescence. It is contemplated that the probe sequences of the present teachings may be labeled with any suitable fluorophore and quencher combinations. For example, any fluorophore of the present teachings may be attached to any probe DNA sequence of the present teachings.
(74) The one or more primers and/or probes maybe selected from the group consisting of: TGGCCAGAACTGACAGGCAAA, TTTCTCCTGAACGTGGCTGGC, 56-FAM/ACGCTAACT/ZEN/CCAGCATTGGTCTGT/3IABkFQ/, CCGTCACGCTGTTGTTAGG, GCTGTGTTAATCAATGCCACAC, 5HEX/AACTTGCCG/ZEN/AATTAGAGCRGCAGT/3IABkFQ, CGTTTCGTCTGGATCGCAC, GCTGGGTAAAATAGGTCACC, 5TEX615/TATCATTGGTGGTGCCGTAGTCGC/3IAbRQSp, GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC, 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp, AATCACAGGGCGTAGTTGTG, ACCCACCAGCCAATCTTAGG, 56-FAM/TAGCTTGAT/ZEN/CGCCCTCGATTTGGG/3IABkFQ/, GCGGAGTTAACTATTGGCTAG, GGCCAAGCTTCTATATTTGCG, 5HEX/TTRTTYGGT/ZEN/GGTTGYTTTRTTAA/3IABkFQ, GCGGAGTTARYTATTGGCTAG, GGCCAAGCYTCTAWATTTGCG, /5HEX/CCGGACGGT/ZEN/CTTGGTAATTTGGGT/3IABkFQ/, /5HEX/CCGTACGGT/ZEN/TTAGGCAATTTGGGT/3IABkFQ/, GGCGGCGTTGATGTCCTTCG, CCATTCAGCCAGATCGGCATC, 5TEX615/AGCTCTTCTATCCTGGTGCTGCG/3IAbRQSp, AACTTTCACAGGTGTGCTGGGT, CCGTACGCATACTGGCTTTGC, 56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IABkFQ/, GTATCGCCGTCTAGTTCTGC, CCTTGAATGAGCTGCACAGTGG, 5HEX/TCGTCGCGG/ZEN/AACCATTCGCTAAA/3IABkFQ/, GTTTGATCGTCAGGGATGGC, GGCGAAAGTCAGGCTGTG, 5TEX615/CATCAGGACAAGATGGGCGGTATG/3IAbRQSp, GCTGCTCAAGGAGCACAGGAT, CACATTGACATAGGTGTGGTGC, 56-FAM/AGGATGGCA/ZEN/AGGCCCACTATTTCA/3IABkFQ, AACAGCCTCAGCAGCCGGTTA, TTCGCCGCAATCATCCCTAGC, 5HEX/AGCCATTAC/ZEN/GTTCCAGAGTTGCGT/3IABkFQ, GCCGAGGCTTACGGGATCAAG, CAAAGCGCGTAACCGGATTGG, 5TEX615/TCTGCTGAAGTTTRYCGAGGCMAA/3IAbRQSp, AACTTTCACAGGTGTGCTGGGT, CCGTACGCATACTGGCTTTGC, 56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IABkFQ/, CTGGGTTCTATAAGTAAAACCTTCACCGG, CTTCCACTGCGGCTGCCAGTT, 5HEX/GATGCCATT/ZEN/GCYCGSGGTGAAAT/3IABkFQ, CCGAAGCCTATGGCGTGAAATCC, GCAATGCCCTGCTGGAGCG, 5TEX615/ATGTTGGCCTGAACCCAGCG/3IAbRQSp. Primers and/or probes included in this group may or may not be degenerate at any nucleotide position. [SEQ. ID NOS 67-118]
(75) The kit may include one or more primer-probe multiplex mixes. The primer-probe multiplex mix may include one or more internal controls. The primer-probe multiplex mix and one or more internal controls may be enclosed in one container, such as a vial. The primer-probe multiplex mix

and one or more internal controls may be enclosed in more than one container, such as vials.
(76) A primer-probe mix may include sequences for detecting any combination of the following genes: CMY-2-like, CTX-M-14-like, CTX-M-15-like, IMP-like, VIM-like, DHA-like, KPC-like, NDM-like, MOX-like, ACC-like, FOX-like, DHA-like, EBC-like, OXA-143-like, OXA-23-like, OXA-51-like, OXA-48-like, OXA-58-like and OXA-24/40-like.
(77) For example, the kit may include a first primer-probe mix and one or more internal controls in a first vial and a second primer-probe mix and one or more internal controls in a second vial. For example, the kit may include a first primer-probe mix and one or more internal controls in a first vial, a second primer-probe mix and one or more internal controls in a second vial and a third primer-probe mix and one or more internal controls in a third vial. Each vial may contain different mixtures. Each vial may contain the same mixture.
(78) The kit may include at least one control DNA mix. The kit may include one or more DNA control mixes. The kit may include exactly two control DNA mixes. The kit may include exactly three control DNA mixes. The DNA control mix may include at least one DNA sequence corresponding to at least one gene family and at least one internal control DNA sequence. The DNA control mix may be enclosed in one container, such as a vial. The DNA control mix may be enclosed in more than one container, such as vials.
(79) For example, the kit may include a first DNA control mix in a first vial and a second DNA control mix in a second vial. For example, the kit may include a first DNA control mix in a first vial, a second DNA control mix in a second vial and a third DNA control mix in a third vial. Each vial may contain different mixtures. Each vial may contain the same mixture.
(80) In one example, the kit includes three primer-probe multiplex mix vials including internal controls and three DNA control mix vials. The three primer-probe multiplex mixes may provide for identification of up to nine antibiotic resistance genes and internal controls. A first primer-probe mix may include sequences for detecting gene families which are CMY-2-like, CTX-M-14-like, CTX-M-15-like and internal controls. A second primer-probe mix may include sequences for detecting gene families which are OXA-48-like, IMP-like, VIM-like and internal controls. A third primer-probe mix may include sequences for detecting gene families which are DHA-like, KPC-like, NDM-like and internal controls. The one or more DNA control mixes may be plasmid or vector controls. A first DNA control mix may include DNA sequences for CMY-2, CTX-M-14, CTX-M-15 and an internal control DNA sequence. A second DNA control mix may include DNA sequences for OXA-48, IMP, VIM and an internal control DNA sequence. A third DNA control mix may include DNA sequences for DHA, KPC, NDM and an internal control DNA sequence.
(81) It is contemplated that the combination of gene families may vary. For example, a primer-probe mix may include sequences for detecting any combination of the following genes: CMY-2-like, CTX-M-14-like, CTX-M-15-like, and OXA-48-like, IMP-like, VIM-like, DHA-like, KPC-like and NDM-like. It is further contemplated that additional β-lactamase gene targets may be included in the primer-probe mix or mixes.
(82) The first primer-probe mix may include one or more primers and/or probes selected from the group consisting of: TGGCCAGAACTGACAGGCAAA, TTTCTCCTGAACGTGGCTGGC, 56FAM/ACGCTAACT/ZEN/CCAGCATTGGTCTGT/3IABkFQ/, CCGTCACGCTGTTGTTAGG, GCTGTGTTAATCAATGCCACAC, 5HEX/AACTTGCCG/ZEN/AATTAGAGCRGCAGT/3IABkFQ, CGTTTCGTCTGGATCGCAC, GCTGGGTAAAATAGGTCACC and 5TEX615/TATCATTGGTGGTGCCGTAGTCGC/3IAbRQSp. The first primer-probe mix may include one or more internal controls selected from the group consisting of: GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC and 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp. A primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix.

[SEQ. ID NOS 152-163]
(83) The kit may include a first, second and third primer and/or probe mix, the first primer and/or probe mix including one or more primers and/or probes selected from the group consisting of: TGGCCAGAACTGACAGGCAAA, TTTCTCCTGAACGTGGCTGGC, 56-FAM/ACGCTAACT/ZEN/CCAGCATTGGTCTGT/3IABkFQ/, CCGTCACGCTGTTGTTAGG, GCTGTGTTAATCAATGCCACAC,
5HEX/AACTTGCCG/ZEN/AATTAGAGCRGCAGT/3IABkFQ, CGTTTCGTCTGGATCGCAC, GCTGGGTAAAATAGGTCACC, 5TEX615/TATCATTGGTGGTGCCGTAGTCGC/3IAbRQSp, GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC, and
5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp. Primers and/or probes included in this group may or may not be degenerate at any nucleotide position. [SEQ. ID NOS 152-163]
(84) The second primer-probe mix may include one or more primers and/or probes selected from the group consisting of: AATCACAGGGCGTAGTTGTG, ACCCACCAGCCAATCTTAGG, 56-FAM/TAGCTTGAT/ZEN/CGCCCTCGATTTGGG/3IABkFQ/,
GCGGAGTTAACTATTGGCTAG, GGCCAAGCTTCTATATTTGCG,
5HEX/TTRTTYGGT/ZEN/GGTTGYTTTRTTAA/3IABkFQ, GCGGAGTTARYTATTGGCTAG, GGCCAAGCYTCTAWATTTGCG,
/5HEX/CCGGACGGT/ZEN/CTTGGTAATTTGGGT/3IABkFQ/,
/5HEX/CCGTACGGT/ZEN/TTAGGCAATTTGGGT/3IABkFQ,
GGCGGCGTTGATGTCCTTCG, CCATTCAGCCAGATCGGCATC and
5TEX615/AGCTCTTCTATCCTGGTGCTGCG/3IAbRQSp. The second primer-probe mix may include one or more internal controls selected from the group consisting of:
GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC, and
5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp. A primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix.
[SEQ. ID NOS 164-179]
(85) The kit may include a first, second, and third primer and/or probe mix, the second primer and/or probe mix including one or more primers and/or probes selected from the group consisting of: AATCACAGGGCGTAGTTGTG, ACCCACCAGCCAATCTTAGG, 56-FAM/TAGCTTGAT/ZEN/CGCCCTCGATTTGGG/13IABkFQ/,
GCGGAGTTAACTATTGGCTAG, GGCCAAGCTTCTATATTTGCG,
5HEX/TTRTTYGGT/ZEN/GGTTGYTTTRTTAA/3IABkFQ, GCGGAGTTARYTATTGGCTAG, GGCCAAGCYTCTAWATTTGCG,
/5HEX/CCGGACGGT/ZEN/CTTGGTAATTTGGGT/3IABkFQ/,
/5HEX/CCGTACGGT/ZEN/TTAGGCAATTTGGGT/3IABkFQ,
GGCGGCGTTGATGTCCTTCG, CCATTCAGCCAGATCGGCATC,
5TEX615/AGCTCTTCTATCCTGGTGCTGCG/3IAbRQSp, GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC, and
5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp. Primers and/or probes included in this group may or may not be degenerate at any nucleotide position. [SEQ. ID NOS 164-179]
(86) The third primer-probe mix may include one or more primers and/or probes selected from the group consisting of: AACTTTCACAGGTGTGCTGGGT, CCGTACGCATACTGGCTTTGC, 56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IABkFQ/, GTATCGCCGTCTAGTTCTGC, CCTTGAATGAGCTGCACAGTGG,
5HEX/TCGTCGCGG/ZEN/AACCATTCGCTAAA/3IABkFQ/, GTTTGATCGTCAGGGATGGC, GGCGAAAGTCAGGCTGTG and
5TEX615/CATCAGGACAAGATGGGCGGTATG/3IAbRQSp. The third primer-probe mix may include one or more internal controls selected from the group consisting of:
GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC and

5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp. A primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix. [SEQ. ID NOS 180-191]

(87) The kit may include a first, second and third primer and/or probe mix, the third primer and/or probe mix including one or more primers and/or probes selected from the group consisting of: AACTTTCACAGGTGTGCTGGGT, CCGTACGCATACTGGCTTTGC, 56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IABkFQ/, GTATCGCCGTCTAGTTCTGC, CCTTGAATGAGCTGCACAGTGG, 5HEX/TCGTCGCGG/ZEN/AACCATTCGCTAAA/3IABkFQ/, GTTTGATCGTCAGGGATGGC, GGCGAAAGTCAGGCTGTG, 5TEX615/CATCAGGACAAGATGGGCGGTATG/3IAbRQSp, GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC, and 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp. Primers and/or probes included in this group may or may not be degenerate at any nucleotide position. [SEQ. ID NOS 180-191]

(88) A first DNA control mix may include one or more sequences selected from the group consisting of:
TGGCCAGAACTGACAGGCAAACAGTGGCAGGGTATCCGCCTGCTGCACTTAGCCA
CCTATACGGCAGGCGGCCTACCGCTGCAGATCCCCGATGACGTTAGGGATAAAGC
CGCATTACTGCATTTTTATCAAAACTGGCAGCCGCAATGGACTCCGGGCGCTAAGC
GACTTTACGCTAACTCCAGCATTGGTCTGTTTGGCGCGCTGGCGGTGAAACCCTC
AGGAATGAGTTACGAAGAGGCAATGACCAGACGCGTCCTGCAACCATTAAAACTG
GCGCATACCTGGATTACGGTTCCGCAGAACGAACAAAAAGATTATGCCTGGGGCT
ATCGCGAAGGGAAGCCCGTACACGTTTCTCCGGGACAACTTGACGCCGAAGCCTA
TGGCGTGAAATCCAGCGTTATTGATATGGCCCGCTGGGTTCAGGCCAACATGGAT
GCCAGCCACGTTCAGGAGAAA,
CCGTCACGCTGTTGTTAGGAAGTGTGCCGCTGTATGCGCAAACGGCGGACGTACA
GCAAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGGGTGTGGCATT
GATTAACACAGC, and
CGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTCCCGGCGACCC
GAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTCAGCTTACGCT
GGGTCATGCGCTGGGCGAAACCCAGCGGGCGCAGTTGGTGACGTGGCTCAAAGG
CAATACGACCGGCGCAGCCAGCATTCGGGCCGGCTTACCGACGTCGTGGACTGT
GGGTGATAAGACCGGCAGCGGCGACTACGGCACCACCAATGATATTGCGGTGATC
TGGCCGCAGGGTCGTGCGCCGCTGGTTCTGGTGACCTATTTTACCCAGC. The first DNA control mix may include the following internal control sequence:
GAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCG. A DNA control mix may include a combination of the one or more said group of sequences and the said internal control sequence. A DNA control mix may include any combination of sequences from the first control mix, the second control mix, the third control mix and the internal control sequence. [SEQ. ID NOS 261-264]

(89) A second DNA control mix may include one or more sequences selected from the group consisting of:
AATCACAGGGCGTAGTTGTGCTCTGGAATGAGAATAAGCAGCAAGGATTTACCAAT
AATCTTAAACGGGCGAACCAAGCATTTTTACCCGCATCTACCTTTAAAATTCCCAAT
AGCTTGATCGCCCTCGATTTGGGCGTGGTTAAGGATGAACACCAAGTCTTTAAGTG
GGATGGACAGACGCGCGATATCGCCACTTGGAATCGCGATCATAATCTAATCACC
GCGATGAAATATTCAGTTGTGCCTGTTTATCAAGAATTTGCCCGCCAAATTGGCGA
GGCACGTATGAGCAAGATGCTACATGCTTTCGATTATGGTAATGAGGACATTTCGG
GCAATGTAGACAGTTTCTGGCTCGACGGTGGTATTCGAATTTCGGCCACGGAGCA
AATCAGCTTTTTAAGAAAGCTGTATCACAATAAGTTACACGTATCGGAGCGCAGCC

AGCGTATTGTCAAACAAGCCATGCTGACCGAAGCCAATGGTGACTATATTATTCGG
GCTAAAACTGGATACTCGACTAGAATCGAACCTAAGATTGGCTGGTGGGT,
GCGGAGTTAGTTATTGGCTAGTTAAAAATAAAATTGAAGTTTTTTATCCCGGCCCGG
GGCACACTCAAGATAACGTAGTGGTTTGGTTACCTGAAAAGAAAATTTTATTCGGT
GGTTGTTTTGTTAAACCGGACGGTCTTGGTAATTTGGGTGACGCAAATTTAGAAGC
TTGGCC and
GGCGGCGTTGATGTCCTTCGGGCGGCTGGGGTGGCAACGTACGCATCACCGTCG
ACACGCCGGCTAGCCGAGGTAGAGGGGAACGAGATTCCCACGCACTCTCTAGAA
GGACTCTCATCGAGCGGGGACGCAGTGCGCTTCGGTCCAGTAGAACTCTTCTATC
CTGGTGCTGCGCATTCGACCGACAACTTAGTTGTGTACGTCCCGTCTGCGAGTGT
GCTCTATGGTGGTTGTGCGATTCATGAGTTGTCACGCACGTCTGCGGGGAACGTG
GCCGATGCCGATCTGGCTGAATGG. The second DNA control mix may include the following internal control sequence:
GAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCG. A DNA control mix may include a combination of the one or more said group of sequences and the said internal control sequence. A DNA control mix may include any combination of sequences from the first control mix, the second control mix, the third control mix and the internal control sequence. [SEQ. ID NOS 265-268]

(90) A third DNA control mix may include one or more sequences selected from the group consisting of:
AACTTTCACAGGTGTGCTGGGTGCGGTTTCTGTGGCGAAAAAAGAGATGGCGCTG
AATGATCCGGCGGCAAAATACCAGCCGGAGCTGGCTCTGCCGCAGTGGAAGGGG
ATCACATTGCTGGATCTGGCTACCTATACCGCAGGCGGACTGCCGTTACAGGTGC
CGGATGCGGTAAAAAGCCGTGCGGATCTGCTGAATTTCTATCAGCAGTGGCAGCC
GTCCCGGAAACCGGGCGATATGCGTCTGTATGCAAACAGCAGTATCGGCCTGTTT
GGTGCTCTGACCGCAAACGCGGCGGGGATGCCGTATGAGCAGTTGCTGACTGCA
CGGATCCTGGCACCGCTGGGGTTATCTCACACCTTTATTACTGTGCCGGAAAGTG
CGCAAAGCCAGTATGCGTACGG,
GTATCGCCGTCTAGTTCTGCTGTCTTGTCTCTCATGGCCGCTGGCTGGCTTTTCTG
CCACCGCGCTGACCAACCTCGTCGCGGAACCATTCGCTAAACTCGAACAGGACTT
TGGCGGCTCCATCGGTGTGTACGCGATGGATACCGGCTCAGGCGCAACTGTAAGT
TACCGCGCTGAGGAGCGCTTCCCACTGTGCAGCTCATTCAAGG and
GTTTGATCGTCAGGGATGGCGGCCGCGTGCTGGTGGTCGATACCGCCTGGACCG
ATGACCAGACCGCCCAGATCCTCAACTGGATCAAGCAGGAGATCAACCTGCCGGT
CGCGCTGGCGGTGGTGACTCACGCGCATCAGGACAAGATGGGCGGTATGGACGC
GCTGCATGCGGCGGGGATTGCGACTTATGCCAATGCGTTGTCGAACCAGCTTGCC
CCGCAAGAGGGGATGGTTGCGGCGCAACACAGCCTGACTTTCGCC. The third DNA control mix may include the following internal control sequence:
GAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCG. A DNA control mix may include a combination of the one or more said group of sequences and the said internal control sequence. A DNA control mix may include any combination of sequences from the first control mix, the second control mix, the third control mix and the internal control sequence. [SEQ. ID NOS 269-272]

(91) In one example, the kit includes two primer-probe multiplex mix vials including internal controls and two DNA control mix vials. The two primer-probe multiplex mixes may provide for identification of up to six antibiotic resistance genes and internal controls. A first primer-probe mix may include sequences for detecting gene families which are MOX-like, ACC-like, FOX-like and internal controls. A second primer-probe mix may include sequences for detecting gene families which are DHA-like, ACT/MIR-like, CMY-2-like and internal controls. A first DNA control mix may include DNA sequences for MOX, ACC, FOX and an internal control DNA sequence. A

second DNA control mix may include DNA sequences for DHA, ACT/MIR, CMY-2 and an internal control DNA sequence.

(92) It is contemplated that the combination of gene families may vary. For example, a primer-probe mix may include sequences for detecting any combination of the following genes: MOX-like, ACC-like, FOX-like, DHA-like, ACT/MIR-like and CMY-2-like. It is further contemplated that additional β-lactamase gene targets may be included in the primer-probe mix or mixes.

(93) The first primer-probe mix may include one or more primers and/or probes selected from the group consisting of: GCTGCTCAAGGAGCACAGGAT, CACATTGACATAGGTGTGGTGC, 56-FAM/AGGATGGCA/ZEN/AGGCCCACTATTTCA/3IABkFQ, AACAGCCTCAGCAGCCGGTTA, TTCGCCGCAATCATCCCTAGC, 5HEX/AGCCATTAC/ZEN/GTTCCAGAGTTGCGT/3IABkFQ, GCCGAGGCTTACGGGATCAAG, CAAAGCGCGTAACCGGATTGG and 5TEX615/TCTGCTGAAGTTTRYCGAGGCMAA/3IAbRQSp. The first primer-probe mix may include one or more internal controls selected from the group consisting of: GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC and 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp. A primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix. [SEQ. ID NOS 192-203]

(94) The second primer-probe mix may include one or more primers and/or probes selected from the group consisting of: AACTTTCACAGGTGTGCTGGGT, CCGTACGCATACTGGCTTTGC, 56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IABkFQ, CTGGGTTCTATAAGTAAAACCTTCACCGG, CTTCCACTGCGGCTGCCAGTT, 5HEX/GATGCCATT/ZEN/GCYCGSGGTGAAAT/3IABkFQ, CCGAAGCCTATGGCGTGAAATCC, GCAATGCCCTGCTGGAGCG, and 5TEX615/ATGTTGGCCTGAACCCAGCG/3IAbRQSp. The second primer-probe mix may include one or more internal controls selected from the group consisting of: GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC and 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp. A primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix. [SEQ. ID NOS 204-215]

(95) The kit may include exactly two primer and/or probe mixes, a first primer and/or probe mix including one or more primers and/or probes selected from the group consisting of: GCTGCTCAAGGAGCACAGGAT, CACATTGACATAGGTGTGGTGC, 56-FAM/AGGATGGCA/ZEN/AGGCCCACTATTTCA/3IABkFQ, AACAGCCTCAGCAGCCGGTTA, TTCGCCGCAATCATCCCTAGC, 5HEX/AGCCATTAC/ZEN/GTTCCAGAGTTGCGT/3IABkFQ, GCCGAGGCTTACGGGATCAAG, CAAAGCGCGTAACCGGATTGG, 5TEX615/TCTGCTGAAGTTTRYCGAGGCMAA/3IAbRQSp, GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC, and 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp; and a second primer and/or probe mix including one or more primers and/or probes selected from the group consisting of: AACTTTCACAGGTGTGCTGGGT, CCGTACGCATACTGGCTTTGC, 56-FAM/AAACCGGGC/ZEN/GATATGCGTCTGTAT/3IABkFQ, CTGGGTTCTATAAGTAAAACCTTCACCGG, CTTCCACTGCGGCTGCCAGTT, 5HEX/GATGCCATT/ZEN/GCYCGSGGTGAAAT/3IABkFQ, CCGAAGCCTATGGCGTGAAATCC, GCAATGCCCTGCTGGAGCG, 5TEX615/ATGTTGGCCTGAACCCAGCG/3IAbRQSp, GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC, and

5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp. Primers and/or probes included in this group may or may not be degenerate at any nucleotide position. [SEQ. ID NOS 192-215]

(96) A first DNA control mix may include one or more sequences selected from the group consisting of:

GCTGCTCAAGGAGCACAGGATCCCGGGCATGGCGGTGGCCGTGCTCAAGGATGG
CAAGGCCCACTATTTCAATTACGGGGTGGCCAACCGGGAGAGCGGGGCCAGCGT
CAGCGAGCAGACCCTGTTCGAGATAGGATCCGTGAGCAAGACCCTGACTGCGACC
CTGGGGGCCTATGCGGTGGTCAAGGGAGCGATGCAGCTGGATGACAAGGCGAGC
CGGCACGCGCCCTGGCTCAAGGGATCCGTCTTTGACAGCATCACCATGGGGGAG
CTTGCCACCTACAGCGCCGGAGGCCTGCCACTGCAATTCCCCGAGGAGGTGGATT
CATCCGAGAAGATGCGCGCCTACTACCGCCAGTGGGCCCCTGTCTATTCGCCGGG
CTCCCATCGCCAGTACTCCAACCCCAGCATAGGGCTGTTCGGCCACCTGGCGGCG
AGCAGCCTGAAGCAGCCATTTGCCCAGTTGATGGAGCAGACCCTGCTGCCCGGG
CTCGGCATGCACCACACCTATGTCAATGTG,

AACAGCCTCAGCAGCCGGTTACGGAAAATACGTTATTTGAAGTGGGTTCGCTGAGT
AAAACGTTTGCTGCCACCTTGGCGTCCTATGCGCAGGTGAGCGGTAAGCTGTCTTT
GGATCAAAGCGTTAGCCATTACGTTCCAGAGTTGCGTGGCAGCAGCTTTGACCAC
GTTAGCGTACTCAATGTGGGCACGCATACCTCAGGCCTACAGCTATTTATGCCGGA
AGATATTAAAAATACCACACAGCTGATGGCTTATCTAAAAGCATGGAAACCTGCCG
ATGCGGCTGGAACCCATCGCGTTTATTCCAATATCGGTACTGGTTTGCTAGGGATG
ATTGCGGCGAA and

GCCGAGGCTTACGGGATCAAGACCGGCTCGGCGGATCTGCTGAAGTTTACCGAG
GCCAACATGGGGTATCAGGGAGATGCCGCGCTAAAAACGCGGATCGCGCTGACC
CATACCGGTTTCTACTCGGTGGGAGACATGACTCAGGGGCTGGGTTGGGAGAGCT
ACGCCTATCCGTTGACCGAGCAGGCGCTGCTGGCGGGCAACTCCCCGGCGGTGA
GCTTCCAGGCCAATCCGGTTACGCGCTTTG. The first DNA control mix may include the following internal control sequence:

GAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCG. A DNA control mix may include a combination of the one or more said group of sequences and the said internal control sequence. A DNA control mix may include any combination of sequences from the first control mix, the second control mix and the internal control sequence. [SEQ. ID NOS 273-276]

(97) A second DNA control mix may include one or more sequences selected from the group consisting of:

AACTTTCACAGGTGTGCTGGGTGCGGTTTCTGTGGCGAAAAAAGAGATGGCGCTG
AATGATCCGGCGGCAAAATACCAGCCGGAGCTGGCTCTGCCGCAGTGGAAGGGG
ATCACATTGCTGGATCTGGCTACCTATACCGCAGGCGGACTGCCGTTACAGGTGC
CGGATGCGGTAAAAAGCCGTGCGGATCTGCTGAATTTCTATCAGCAGTGGCAGCC
GTCCCGGAAACCGGGCGATATGCGTCTGTATGCAAACAGCAGTATCGGCCTGTTT
GGTGCTCTGACCGCAAACGCGGCGGGGATGCCGTATGAGCAGTTGCTGACTGCA
CGGATCCTGGCACCGCTGGGGTTATCTCACACCTTTATTACTGTGCCGGAAAGTG
CGCAAAGCCAGTATGCGTACGG,

TCGGTAAAGCCGATGTTGCGGCGAACAAACCCGTCACCCCGCAAACCCTGTTTGA
GCTGGGCTCTATAAGTAAAACCTTCACCGGCGTACTGGGCGGCGATGCCATTGCC
CGGGGTGAAATAGCGCTGGGCGATCCGGTAGCAAAATACTGGCCTGAGCTCACG
GGCAAGCAGTGGCAGGGCATTCGCATGCTGGATCTGGCAACCTATACCGCAGGC
GGTCTGCCGTTACAGGTGCCGGATGAGGTCACGGATACCGCCTCTCTGCTGCGCT
TTTATCAAAACTGGCAGCCGCAGTGGAAG and

CCGAAGCCTATGGCGTGAAATCCAGCGTTATTGATATGGCCCGCTGGGTTCAGGC
CAACATGGATGCCAGCCACGTTCAGGAGAAAACGCTCCAGCAGGGCATTGC. The

second DNA control mix may include the following internal control sequence: GAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG GCAGCAGTGGGGAATATTGCACAATGGGCG. A DNA control mix may include a combination of the one or more said group of sequences and the said internal control sequence. A DNA control mix may include any combination of sequences from the first control mix, the second control mix and the internal control sequence. [SEQ. ID NOS 277-280]

(98) In one example, the kit includes two primer-probe multiplex mix vials including internal controls and two DNA control mix vials. The two primer-probe multiplex mixes may provide for identification of up to six antibiotic resistance genes and internal controls. A first primer-probe mix may include sequences for detecting gene families which are OXA-143-like, OXA-23-like, OXA-51-like and internal controls. A second primer-probe mix may include sequences for detecting gene families which are OXA-48-like, OXA-58-like, OXA-24/40-like and internal controls. A first DNA control mix may include DNA sequences for OXA-143, OXA-23, OXA-51 and an internal control DNA sequence. A second DNA control mix may include DNA sequences for OXA-48, OXA-58 and OXA 24/40 and an internal control DNA sequence.

(99) It is contemplated that the combination of gene families may vary. For example, a primer-probe mix may include sequences for detecting any combination of the following genes: OXA-143-like, OXA-23-like, OXA-51-like, OXA-48-like, OXA-58-like and OXA-24/40-like. It is further contemplated that additional β-lactamase gene targets may be included in the primer-probe mix or mixes.

(100) The first primer-probe mix may include one or more primers and/or probes selected from the group consisting of: AGCACATACAGAATATGTCCCTGC, ACCTGTTAACCAACCTACTTGAGGG, /56-FAM/TTGCAAGACGGACTGGCTTAGACC/3BHQ_1/, CCTGATCGGATTGGAGAACC, CTACCTCTTGAATAGGCGTAACC, /5TEX615/ACGTCGCGCAAGTTCCTGATAGAC/3IAbRQSp/, TAGTGACTGCTAATCCAAATCACAG, GCACGAGCAAGATCATTACCATAGC, /5HEX/AGTTATCCAACAAGGCCAAACTCAACA/3BHQ_1/. [SEQ. ID NOS 119-127] The first primer-probe mix may include one or more internal controls selected from the group consisting of: GAGAGGATGAYCAGCCACAC (SEQ ID NO: 201), CGCCCATTGTSCAATATTCC (SEQ ID NO: 202) and 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp (SEQ ID NO: 203). A primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix.

(101) The second primer-probe mix may include one or more primers and/or probes selected from the group consisting of: AATCACAGGGCGTAGTTGTG, ACCCACCAGCCAATCTTAGG, /5HEX/TAGCTTGATCGCCCTCGATTTGGG/3BHQ_1/, GTGGGATGGAAAGCCACG, CACTTGCGGGTCTACAGC, /56-FAM/TTACTTTGGGCGAAGCCATGCAAG/3BHQ_1/, CACCTATGGTAATGCTCTTGC, CTGGAACTGCTGACAATGCC, /5TEX615/TGGGAGAAAGATATGACTTTAGGTGAGGCA/3IAbRQSp/. [SEQ. ID NOS 128-136] The second primer-probe mix may include one or more internal controls selected from the group consisting of: GAGAGGATGAYCAGCCACAC (SEQ ID NO: 201), CGCCCATTGTSCAATATTCC (SEQ ID NO: 202) and 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp (SEQ ID NO: 203). A primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix.

(102) The kit may include exactly two primer and/or probe mixes, a first primer and/or probe mix including one or more primers and/or probes selected from the group consisting of: AGCACATACAGAATATGTCCCTGC, ACCTGTTAACCAACCTACTTGAGGG, /56-

FAM/TTGCAAGACGGACTGGCTTAGACC/3BHQ_1/, CCTGATCGGATTGGAGAACC, CTACCTCTTGAATAGGCGTAACC, /5TEX615/ACGTCGCGCAAGTTCCTGATAGAC/3IAbRQSp/, TAGTGACTGCTAATCCAAATCACAG, GCACGAGCAAGATCATTACCATAGC, /5HEX/AGTTATCCAACAAGGCCAAACTCAACA/3BHQ_1/, GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC and 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp; and a second primer and/or probe mix including one or more primers and/or probes selected from the group consisting of: AATCACAGGGCGTAGTTGTG, ACCCACCAGCCAATCTTAGG, /5HEX/TAGCTTGATCGCCCTCGATTTGGG/3BHQ_1/, GTGGGATGGAAAGCCACG, CACTTGCGGGTCTACAGC, /56-FAM/TTACTTTGGGCGAAGCCATGCAAG/3BHQ_1/, CACCTATGGTAATGCTCTTGC, CTGGAACTGCTGACAATGCC, /5TEX615/TGGGAGAAAGATATGACTTTAGGTGAGGCA/3IAbRQSp/, GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC and 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp. Primers and/or probes included in this group may or may not be degenerate at any nucleotide position. [SEQ. ID NOS 216-239]

(103) A first DNA control mix may include one or more sequences selected from the group consisting of:
AGCACATACAGAATATGTCCCTGCATCAACATTTAAGATGCTAAATGCCTTAATTGG
ACTAGAAAATCATAAAGCTACAACAACTGAGATTTTCAAATGGGACGGTAAAAAGA
GATCTTATCCCATGTGGGAAAAAGATATGACTTTAGGTGATGCCATGGCACTTTCA
GCAGTTCCTGTATATCAAGAACTTGCAAGACGGACTGGCTTAGACCTAATGCAAAA
AGAAGTTAAACGGGTTGGTTTTGGTAATATGAACATTGGAACACAAGTTGATAACTT
CTGGTTGGTTGGCCCCCTCAAGATTACACCAATACAAGAGGTTAATTTTGCCGATG
ATTTTGCAAATAATCGATTACCCTTTAAATTAGAGACTCAAGAAGAAGTTAAAAAAAT
GCTTCTGATTAAAGAATTCAATGGTAGTAAAATTTATGCAAAAAGCGGCTGGGGAA
TGGATGTAACCCCTCAAGTAGGTTGGTTAACAGGT,
CCTGATCGGATTGGAGAACCAGAAAACGGATATTAATGAAATATTTAAATGGAAGG
GCGAGAAAAGGTCATTTACCGCTTGGGAAAAAGACATGACACTAGGAGAAGCCAT
GAAGCTTTCTGCAGTCCCAGTCTATCAGGAACTTGCGCGACGTATCGGTCTTGATC
TCATGCAAAAAGAAGTAAAACGTATTGGTTTCGGTAATGCTGAAATTGGACAGCAG
GTTGATAATTTCTGGTTGGTAGGACCATTAAAGGTTACGCCTATTCAAGAGGTAG and
TAGTGACTGCTAATCCAAATCACAGCGCTTCAAAATCTGATGAAAAAGCAGAGAAA
ATTAAAAATTTATTTAACGAAGTACACACTACGGGTGTTTTAGTTATCCAACAAGGC
CAAACTCAACAAAGCTATGGTAATGATCTTGCTCGTGC. The first DNA control mix may include the following internal control sequence:
GAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCG. A DNA control mix may include a combination of the one or more said group of sequences and the said internal control sequence. A DNA control mix may include any combination of sequences from the first control mix, the second control mix and the internal control sequence. [SEQ. ID NOS 281-284]

(104) A second DNA control mix may include one or more sequences selected from the group consisting of:
AATCACAGGGCGTAGTTGTGCTCTGGAATGAGAATAAGCAGCAAGGATTTACCAAT
AATCTTAAACGGGCGAACCAAGCATTTTTACCCGCATCTACCTTTAAAATTCCCAAT
AGCTTGATCGCCCTCGATTTGGGCGTGGTTAAGGATGAACACCAAGTCTTTAAGTG
GGATGGACAGACGCGCGATATCGCCACTTGGAATCGCGATCATAATCTAATCACC
GCGATGAAATATTCAGTTGTGCCTGTTTATCAAGAATTTGCCCGCCAAATTGGCGA
GGCACGTATGAGCAAGATGCTACATGCTTTCGATTATGGTAATGAGGACATTTCGG
GCAATGTAGACAGTTTCTGGCTCGACGGTGGTATTCGAATTTCGGCCACGGAGCA

AATCAGCTTTTTAAGAAAGCTGTATCACAATAAGTTACACGTATCGGAGCGCAGCC
AGCGTATTGTCAAACAAGCCATGCTGACCGAAGCCAATGGTGACTATATTATTCGG
GCTAAAACTGGATACTCGACTAGAATCGAACCTAAGATTGGCTGGTGGGT,
GTGGGATGGAAAGCCACGTTTTTTTAAAGCATGGGACAAAGATTTTACTTTGGGCG
AAGCCATGCAAGCATCTACAGTGCCTGTATATCAAGAATTGGCACGTCGTATTGGT
CCAAGCTTAATGCAAAGTGAATTGCAACGTATTGGTTATGGCAATATGCAAATAGG
CACGGAAGTTGATCAATTTTGGTTGAAAGGGCCTTTGACAATTACACCTATACAAG
AAGTAAAGTTTGTGTATGATTTAGCCCAAGGGCAATTGCCTTTTAAACCTGAAGTTC
AGCAACAAGTGAAAGAGATGTTGTATGTAGAGCGCAGAGGGGAGAATCGTCTATA
TGCTAAAAGTGGCTGGGGAATGGCTGTAGACCCGCAAGTG,
CACTTGCGGGTCTACAGCCATTCCCCAGCCACTTTTAGCATATAGACGATTCTCCC
CTCTGCGCTCTACATACAACATCTCTTTCACTTGTTGCTGAACTTCAGGTTTAAAAG
GCAATTGCCCTTGGGCTAAATCATACACAAACTTTACTTCTTGTATAGGTGTAATTG
TCAAAGGCCCTTTCAACCAAAATTGATCAACTTCCGTGCCTATTTGCATATTGCCAT
AACCAATACGTTGCAATTCACTTTGCATTAAGCTTGGACCAATACGACGTGCCAATT
CTTGATATACAGGCACTGTAGATGCTTGCATGGCTTCGCCCAAAGTAAAATCTTTGT
CCCATGCTTTAAAAAAACGTGGCTTTCCATCCCAC, and
CACCTATGGTAATGCTCTTGCACGAGCAAATAAAGAATATGTCCCTGCATCAACATT
TAAGATGCTAAATGCTTTAATCGGGCTAGAAAATCATAAAGCAACAACAAATGAGAT
TTTCAAATGGGATGGTAAAAAAAGAACTTATCCTATGTGGGAGAAAGATATGACTTT
AGGTGAGGCAATGGCATTGTCAGCAGTTCCAG. The second DNA control mix may include the following internal control sequence:
GAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCG. A DNA control mix may include a combination of the one or more said group of sequences and the said internal control sequence additional β-lactamase. A DNA control mix may include any combination of sequences from the first control mix, the second control mix and the internal control sequence. [SEQ. ID NOS 285-289]

(105) In one example, the kit includes one primer-probe multiplex mix vials including internal control and one DNA control mix vial. A primer-probe mix may include sequences for detecting MCR gene families and internal control.

(106) The primer-probe mix may include primers and/or probes selected from the group consisting of: CCGTGTATGTTCAGCTAT, CTTATCCATCACGCCTTT,
/5TEX615/TATGATGTCGATACCGCCAAATACCA/3IAbRQSp/, CTGTATGTCAGCGATCAT,
GATGCCAGTTTGCTTATCC, /56-
FAM/AAGTCTGGG/ZEN/TGAGAACGGTGTCTAT/3IABkFQ/, CAGTCAGTATGCGAGTTTC,
AAAATTCGCCAAGCCATC, and
/5HEX/TGCATAAGC/ZEN/CAGTGCGTTTTTATAT/3IABkFQ/. The primer-probe mix may include one or more internal controls selected from the group consisting of:
GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC and
5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp. The primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix. [SEQ. ID NOS 137-145]

(107) A DNA control mix may include one or more sequences selected from the group consisting of

(108) TABLE-US-00002
ATGATGCAGCATACTTCTGTGTGGTACCGACGCTCGGTCAGTCCGTTTGT
TCTTGTGGGAGTGTTGCCGTTTTCTTGACCGCGACCGCCAATCTTACCTT
TTTTGATAAAATCAGCCAAACCTATCCCATCGCGGACAATCTCGGCTTTG
TGCTGACGATCGCTGTCGTGCTCTTTGGCGCGATGCTACTGATCACCACG

CTGTTATCATCGTATCGCTATGTGCTAAAGCCTGTGTTGATTTTGCTATT
AATCATGGGCGCGGTGACCAGTTATTTTACTGACACTTATGGCACGGTCT
ATGATACGACCATGCTCCAAAATGCCCTACAGACCGACCAAGCCGAGACC
AAGGATCTATTAAACGCAGCGTTTATCATGCGTATCATTGGTTTGGGTGT
GCTACCAAGTTTGCTTGTGGCTTTTGTTAAGGTGGATTATCCGACTTGGG
GCAAGGGTTTGATGCGCCGATTGGGCTTGATCGTGGCAAGTCTTGCGCTG
ATTTTACTGCCTGTGGTGGCGTTCAGCAGTCATTATGCCAGTTTCTTTCG
CGTGCATAAGCCGCTGCGTAGCTATGTCAATCCGATCATGCCAATCTACT
CGGTGGGTAAGCTTGCCAGTATTGAGTATAAAAAAGCCAGTGCGCCAAAA
GATACCATTTATCACGCCAAAGACGCGGTACAAGCAACCAAGCCTGATAT
GCGTAAGCCACGCCTAGTGGTGTTCGTCGTCGGTGAGACGGCACGCGCCG
ATCATGTCAGCTTCAATGGCTATGAGCGCGATACTTTCCCACAGCTTGCC
AAGATCGATGGCGTGACCAATTTTAGCAATGTCACATCGTGCGGCACATC
GACGGCGTATTCTGTGCCGTGTATGTTCAGCTATCTGGGCGCGGATGAGT
ATGATGTCGATACCGCCAAATACCAAGAAAATGTGCTGGATACGCTGGAT
CGCTTGGGCGTAAGTATCTTGTGGCGTGATAATAATTCGGACTCAAAAGG
CGTGATGGATAAGCTGCCAAAAGCGCAATTTGCCGATTATAAATCCGCGA
CCAACAACGCCATCTGCAACACCAATCCTTATAACGAATGCCGCGATGTC
GGTATGCTCGTTGGCTTAGATGACTTTGTCGCTGCCAATAACGGCAAAGA
TATGCTGATCATGCTGCACCAAATGGGCAATCACGGGCCTGCGTATTTTA
AGCGATATGATGAAAAGTTTGCCAAATTCACGCCAGTGTGTGAAGGTAAT
GAGCTTGCCAAGTGCGAACATCAGTCCTTGATCAATGCTTATGACAATGC
CTTGCTTGCCACCGATGATTTCATCGCTCAAAGTATCCAGTGGCTGCAGA
CGCACAGCAATGCCTATGATGTCTCAATGCTGTATGTCAGCGATCATGGC
GAAAGTCTGGGTGAGAACGGTGTCTATCTACATGGTATGCCAAATGCCTT
TGCACCAAAAGAACAGCGCAGTGTGCCTGCATTTTTCTGGACGGATAAGC
AAACTGGCATCACGCCAATGGCAACCGATACCGTCCTGACCCATGACGCG
ATCACGCCGACATTATTAAAGCTGTTTGATGTCACCGCGGACAAAGTCAA
AGACCGCACCGCATTCATCCGCTGA and
ATGACATCACATCACTCTTGGTATCGCTATTCTATCAATCCTTTTGTGCT
GATGGGTTTGGTGGCGTTATTTTTGGCAGCGACAGCGAACCTGACATTTT
TTGAAAAAGCGATGGCGGTCTATCCTGTATCGGATAACTTAGGCTTTATC
ATCTCAATGGCGGTGGCGGTGATGGGTGCTATGCTACTGATTGTCGTGCT
GTTATCCTATCGCTATGTGCTAAAGCCTGTCCTGATTTTGCTACTGATTA
TGGGTGCGGTGACGAGCTATTTTACCGATACTTATGGCACGGTCTATGAC
ACCACCATGCTCCAAAATGCCATGCAAACCGACCAAGCCGAGTCTAAGGA
CTTGATGAATTTGGCGTTTTTTGTGCGAATTATCGGGCTTGGCGTGTTGC
CAAGTGTGTTGGTCGCAGTTGCCAAAGTCAATTATCCAACATGGGGCAAA
GGTCTGATTCAGCGTGCGATGACATGGGGTGTCAGCCTTGTGCTGTTGCT
TGTGCCGATTGGACTATTTAGCAGTCAGTATGCGAGTTTCTTTCGGGTGC
ATAAGCCAGTGCGTTTTTATATCAACCCGATTACGCCGATTTATTCGGTG
GGTAAGCTTGCCAGTATCGAGTACAAAAAAGCCACTGCGCCAACAGACAC
CATCTATCATGCCAAAGACGCCGTGCAGACCACCAAGCCGAGCGAGCGTA
AGCCACGCCTAGTGGTGTTCGTCGTCGGTGAGACGGCGCGTGCTGACCAT
GTGCAGTTCAATGGCTATGGCCGTGAGACTTTCCCGCAGCTTGCCAAAGT
TGATGGCTTGGCGAATTTTAGCCAAGTGACATCGTGTGGCACATCGACGG
CGTATTCTGTGCCGTGTATGTTCAGCTATTTGGGTCAAGATGACTATGAT
GTCGATACCGCCAAATACCAAGAAAATGTGCTAGATACGCTTGACCGCTT
GGGTGTGGGTATCTTGTGGCGTGATAATAATTCAGACTCAAAAGGCGTGA
TGGATAAGCTACCTGCCACGCAGTATTTTGATTATAAATCAGCAACCAAC

AATACCATCTGTAACACCAATCCCTATAACGAATGCCGTGATGTCGGTAT
GCTTGTCGGGCTAGATGACTATGTCAGCGCCAATAATGGCAAAGATATGC
TCATCATGCTACACCAAATGGGCAATCATGGGCCGGCGTACTTTAAGCGT
TATGATGAGCAATTTGCCAAATTCACCCCCGTGTGCGAAGGCAACGAGCT
TGCCAAATGCGAACACCAATCACTCATCAATGCCTATGACAATGCGCTAC
TTGCGACTGATGATTTTATCGCCAAAAGCATCGATTGGCTAAAAACGCAT
GAAGCGAACTACGATGTCGCCATGCTCTATGTCAGTGACCACGGCGAGAG
CTTGGGCGAAAATGGTGTCTATCTGCATGGTATGCCAAATGCCTTTGCAC
CAAAAGAACAGCGAGCTGTGCCTGCGTTTTTTTGGTCAAATAATACGACA
TTCAAGCCAACTGCCAGCGATACTGTGCTGACGCATGATGCGATTACGCC
AACACTGCTTAAGCTGTTTGATGTCACAGCGGGCAAGGTCAAAGACCGCG
CGGCATTTATCCAGTAA.

The DNA control mix may include the following internal control sequence:
GAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCG. A DNA control mix may include a combination of the one or more said group of sequences and the said internal control sequence. [SEQ. ID NOS 290-292]

(109) In one example, the kit includes one primer-probe multiplex mix vial including internal control and one DNA control mix vial. A primer-probe mix may include sequences for detecting TEM-like and SHV-like gene families and internal control.

(110) The primer-probe mix may include primers and/or probes selected from the group consisting of: AGATCAGTTGGGTGCACG, TGCTTAATCAGTGAGGCACC, /56-FAM/ATGAAGCCA/ZEN/TACCAAACGACGAGC/3IABkFQ/, CTGGAGCGAAAGATCCACTA, ATCGTCCACCATCCACTG, and /5HEX/CCAGATCGG/ZEN/CGACAACGTCACC/3IABkFQ/. The primer-probe mix may include one or more internal controls selected from the group consisting of: GAGAGGATGAYCAGCCACAC, CGCCCATTGTSCAATATTCC and 5TYE665/TGAGACACGGTCCAGACTCCTACG/3IAbRQSp. The primer-probe mix may include a combination of the one or more said group of primers and/or probes and the one or more said group of internal controls. The primer-probe mix including internal controls may be a multiplex mix. [SEQ. ID NOS 240-248]

(111) A DNA control mix may include one or more sequences selected from the group consisting of: AGATCAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAG
ATCCTTGAGAGTTTTCGCCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTT
CTGCTATGTGGTGCGGTATTATCCCGTGTTGACGCCGGGCAAGAGCAACTCGGTC
GCCGCATACACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAG
CATCTTACGGATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAG
TGATAACACTGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTA
ACCGCTTTTTTGCACAACATGGGGGATCATGTAACTCGCCTTGATCGTTGGGAACC
GGAGCTGAATGAAGCCATACCAAACGACGAGCGTGACACCACGACGCCTGCAGC
AATGGCAACAACGTTGCGCAAACTATTAACTGGCGAACTACTTACTCTAGCTTCCC
GGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCG
CTCGGCCCTTCCGGCTGGCTGGTTTATTGCTGATAAATCTGGAGCCAGTGAGCGT
GGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTAAGCCCTCCCGTATCG
TAGTTATCTACACGACGGGGAGTCAGGCAACTATGGATGAACGAAATAGACAGATC
GCTGAGATAGGTGCCTCACTGATTAAGCA and
CTGGAGCGAAAGATCCACTATCGCCAGCAGGATCTGGTGGACTACTCGCCGGTCA
GCGAAAAACACCTTGCCGACGGCATGACGGTCGGCGAACTCTGCGCCGCCGCCA
TTACCATGAGCGATAACAGCGCCGCCAATCTGCTGCTGGCCACCGTCGGCGGCC
CCGCAGGATTGACTGCCTTTTTGCGCCAGATCGGCGACAACGTCACCCGCCTTGA

CCGCTGGGAAACGGAACTGAATGAGGCGCTTCCCGGCGACGCCCGCGACACCAC
TACCCCGGCCAGCATGGCCGCGACCCTGCGCAAGCTGCTGACCAGCCAGCGTCT
GAGCGCCCGTTCGCAACGGCAGCTGCTGCAGTGGATGGTGGACGAT. The DNA control mix may include the following internal control sequence:
GAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTACGGGAG
GCAGCAGTGGGGAATATTGCACAATGGGCG. A DNA control mix may include a combination of the one or more said group of sequences and the said internal control sequence. [SEQ. ID NOS 293-295]

(112) The primer-probe multiplex mix may comprise different oligonucleotide sequences. An oligonucleotide sequence may be utilized as a primer. An oligonucleotide sequence may be utilized as a probe. An oligonucleotide sequence may be utilized as an internal control sequence. The oligonucleotide concentration of a primer and/or probe sequence may range from 0.05 μM to 60 μM. For example, the oligonucleotide concentration of a primer and/or probe sequence may range from 3 μM to 8 μM. For example, the oligonucleotide concentration of an internal control sequence may range from 2 μM to 6 μM. For example, the oligonucleotide concentration of an internal control sequence may range from 2 μM to 8 μM. The vial oligonucleotide concentrations may be prepared as a 10× stock solution.

(113) The synthetic gene size of a DNA control sequence may be from about 84 bp to about 533 bp. The concentration of a DNA control sequence may be about 25 ng/μl. The concentration of a DNA control sequence may be from 0.033 ng/μL to about 0.5 ng/μl.

(114) The present teachings provide methods for detection of β-lactamase gene families from a biological sample. Preferably, the sample includes Gram-negative bacteria. The method may include sample processing. The method may include extracting DNA from the sample. The method may include extracting RNA from the sample. The method may include the use of assays of the present teachings. The assays may be included in a kit or kits. The method may include employing the kit of the present teachings for the detection of multiple β-lactamase gene families from a biological sample.

(115) The method may include employing the kit for analysis of nucleic acid contained in a clinical sample. The method may include employing the kit for analysis of DNA extracted from a clinical sample. The method may include employing the kit for analysis of DNA extracted from an overnight bacterial culture of a clinical sample.

(116) The method may include amplifying a targeted DNA sequence by real-time polymerase reaction. The method may include amplifying several targeted DNA sequences by multiplex real-time polymerase reaction. The method may include analyzing the amplified sequences or amplicons. The method may include detecting the presence or absence of β-lactamase genes. The method may include detecting the presence or absence of ampC β-lactamase genes. The method may include identifying up to six β-lactamase gene families. The method may include identifying up to nine β-lactamase gene families. The method may include identifying up to fifteen β-lactamase gene families. The method may include identifying up to twenty β-lactamase gene families. The method may include identifying from about six to about thirty β-lactamase gene families. The method may include analyzing collected data.

(117) Examples of real-time PCR amplification curves obtained on the ABI QS7 Flex-Real-Time System for some of the multiplex mixes described herein are shown in FIGS. **1**-**9**. FIG. **1** depicts an amplification plot of an exemplary mix 1 including ampC gene targets. FIG. **2** depicts an amplification plot of an exemplary mix 2 including ampC gene targets. FIG. **3** depicts an amplification plot of an exemplary mix 1 including β-lactamase gene targets. FIG. **4** depicts an amplification plot of an exemplary mix 2 including β-lactamase gene targets. FIG. **5** depicts an amplification plot of an exemplary mix 3 including β-lactamase gene targets. FIG. **6** depicts an amplification plot of an exemplary internal control mix including MCR gene targets. FIG. **7** depicts an amplification plot of an exemplary mix 1 including OXA gene targets. FIG. **8** depicts an

amplification plot of an exemplary mix 2 including OXA gene targets. FIG. **9** depicts an amplification plot of an exemplary internal control mix including SHV-TEM gene targets.
(118) The method may include using one or more oligonucleotide primers that are complementary to at least a portion of the nucleic acid sequence of interest. The method may include annealing several pairs of primers to different target DNA sequences. The method may include annealing primer/probe sequences to bacterial nucleic acid sequences comprising targeted antibiotic resistant gene family variants of β-lactamases. The primer and/or probe sequences may anneal with 100% specificity to the target gene variants. The primer and/or probe sequences may anneal with about 95% specificity to the target gene variants. The primer and/or probe sequences may anneal with about 90% to about 100% specificity to the target gene variants. The primer and/or probe sequences may anneal with about 80% to about 100% specificity to the target gene variants.
(119) The method may include using temperature mediated DNA polymerase. The method may include using fluorescent dyes. The method may include the using sequence specific DNA probes including oligonucleotides labeled with a reporter. The method may include using a microarray.
(120) The method may include using a thermal cycler. For example, the kit of the present teachings may be utilized with the following PCR systems: Streck ZULU RT™ PCR System, Applied Biosystems (ABI) QuantStudio 7 (QS7) Flex Real-Time System, ABI 7500 Real-Time PCR System, QIAGEN Rotor-Gene® Q, and CFX96 Touch™ Real-Time PCR Detection System, Applied Biosystems™ 7500 Fast Dx Real-Time PCR Instrument, Roche LightCycler® 480 I and II, and Cepheid SmartCycler®. It is contemplated that any detection system capable of detecting the multiplex fluorescent signal provided in the kit of the present teachings may be suitable.
(121) The method may include real-time monitoring of qPCR reaction products. The probes may generate a signal when hydrolyzed by the DNA polymerase causing liberation of a detectable fluorescent signal. The real-time monitoring method may employ fluorescence at different wavelengths. The method may include the use of DNA-intercalating fluorescent dyes. The method may include the use of a target specific nucleotide probe labeled with a fluorescent tag at one end. The other end of the hybridization probe may be labeled with a fluorescent quencher. Fluorescent hybridization probes generate a fluorescence signal only when they bind to their target and enable real-time of monitoring of nucleic acid amplification assays.
(122) Surprisingly, some DNA targets detected with these kits, allow for amplification of regions of DNA much larger than the conventional wisdom within the real-time PCR field. For example, most amplicons would traditionally be between 50 to 150 base pairs in size. The present teachings allow for successfully amplified amplicons up to 553 base pairs by real-time PCR.
(123) There may be one or more benefits to detecting larger amplicons. Larger amplicons may, in some cases, provide greater specificity for a specific antibiotic resistance gene family. Detection of larger amplicons may permit detection of an increased number of gene variants within a given resistance gene family. Detection of larger amplicons may also allow confirmation by agarose gel electrophoresis since the molecular sizes of each gene that is detected can be resolved from one another.
(124) The efficiency of detection for each target in a dilution series may be measured for amplicons between 25 base pairs and 2000 base pairs. The efficiency of the PCR for amplicons within this size range may be from 80% to 110%. More specifically, the efficiency of the reactions may be from 90% to 105%. The coefficient of determination may be from 0.98 to 1.1. More specifically, the coefficient of determination may be from 0.99 to 1.0. The limit of detection may be from 0.1 copies to 1×10.sup.10 copies.
(125) Alternate sequences for primer, probes, and DNA controls for β-lactamase gene targets of the present teachings are depicted in Table 2 and Table 3. [SEQ. ID NOS 1-48 and SEQ. ID NOS 49-66]
(126) Primers and/or probes may be degenerate at any nucleotide position. Primers and/or probes may not be degenerate at any nucleotide position. Any suitable fluorophore and/or quencher and

nucleic acid sequence combination may be used. For example, a probe may be labeled with a fluorescent tag at one end and a fluorescent quencher at the other end. For example, a probe may be labeled with a fluorescent tag at one end and a fluorescent quencher at the other end. For example, two fluorescent quenchers may be included at one end or within the probe sequence. It is contemplated that the probe sequences of the present teachings may be labeled with any suitable fluorophore and quencher combinations. For example, any fluorophore of the present teachings may be attached to any probe DNA sequence of the present teachings.

(127) TABLE-US-00003 TABLE 2 Primer/ Probe Sequence SEQ ID NO. 1 MOX F′ AGA CCC TGT TCG AGA TAG SEQ ID NO. 2 MOX R′ ATG GTG ATG CTG TCA AAG SEQ ID NO. 3 MOX-FAM 5′-56-FAM-CGT GAG CAA GAC CCT GAC TG-3′BHQ1 SEQ ID NO. 4 FOX F′ ACT ATT TCA ACT ATG GGG TT SEQ ID NO. 5 FOX R′ TTG TCA TCC AGC TCA AAG SEQ ID NO. 6 FOX-TEX 5′-Tex615-TGA CCG CAG CAT AGG CAC-3′BHQ_2 SEQ ID NO. 7 EBC F′ GTG GCG GTG ATT TAT GAG SEQ ID NO. 8 EBC R′ CGG TGA AGG TTT TAC TTA TAG AA SEQ ID NO. 9 EBC-HEX 5′-5HEX/CAGCCGCAC/ZEN/TACTTCACCT/-3′BHQ_1 SEQ ID NO. 10 DHA F′ TGCGTACGGTTATGAGAACAA SEQ ID NO. 11 DHA R′ CCCAGCGCAGCATATCTT SEQ ID NO. 12 DHA-FAM ATGCGGAATCTTACGGCGTGGAAT SEQ ID NO. 13 CMY F′ TCC AGC GTT ATT GAT ATG G SEQ ID NO. 14 CMY R′ CAT CTC CCA GCC TAA TCC SEQ ID NO. 15 CMY-TEX 5′TexRd-XN/ACATATCGCCAATACGCCAGT/3IAPRQSp/-3′ SEQ ID NO. 16 ACC F′ GCCGCTGATGCAGAAGAATA SEQ ID NO. 17 ACC R′ TTT GCC GCT AAC CCA TAG TT SEQ ID NO. 18 ACC-HEX 5′-/5HEX/TCA CTG CGA/ZEN/CCG ACA TAC CG/3IABkFQ/-3′ SEQ ID NO. 19 IC F′ GAG AGG ATG ACC AGC CAC AC SEQ ID NO. 20 IC R′ AGT ACT TTA CAA CCC GAA GGC SEQ ID NO. 21 IC-TYE 5′/5TYE665/TGA GAC ACG GTC CAG ACT CCT ACG G/3BHQ_2/-3′ SEQ ID NO. 22 CTX-M-14 5′-TTGGTGACGTGGCTCAAA-3′ F′ SEQ ID NO. 23 CTX-M-14 5′-ATATCATTGGTGGTGCCGTAG-3′ R′ SEQ ID NO. 24 CTX-M-14- 5′-/56-FAM/CGTGGACTG/ZEN/TGGGTGATAAGACCG/3IABkFQ/-3′ FAM SEQ ID NO. 25 CTX-M-15 5′-GTCACGCTGTTGTTAGGAAGT-3′ F′ SEQ ID NO. 26 CTX-M-15 5′-TAATCAATGCCACACCCAGTC-3′ R′ SEQ ID NO. 27 CTX-M-15- 5′-/5TEX615/AACTTGCCGAATTAGAGCGGCAGT/3BHQ_2/-3′ TEX615 SEQ ID NO. 28 OXA48-F′ 5′-AGCAGCAAGGATTTACCAATAATC-3′ SEQ ID NO. 29 OXA48-R′ 5′-CGTCTGTCCATCCCACTTAAA-3′ SEQ ID NO. 30 OXA48-HEX 5′-/5HEX/TAGCTTGAT/ZEN/CGCCCTCGATTTGGG/3IABkFQ/-3′ SEQ ID NO. 31 CMY F′ 5′-TCCAGCGTTATTGATATGG-3′ SEQ ID NO. 32 CMY R′ 5′-CATCTCCCAGCCTAATCC-3′ SEQ ID NO. 33 CMY-TxR 5-/5TexRd-XN/ACATATCGCCAATACGCCAGT/3IAbRQSp/-3′ SEQ ID NO. 34 NDM F′ 5′-TTTGATCGTCAGGGATGGC-3′ SEQ ID NO. 35 NDM R′ 5′-CAGGTTGATCTCCTGCTTGAT-3′ SEQ ID NO. 36 NDM-HEX 5-/5HEX/AGACCGCCC/ZEN/AGATCCTCAACTG/3IABkFQ/-3′ SEQ ID NO. 37 KPC F′ 5′-CGCTAAACTCGAACAGGACTT-3′ SEQ ID NO. 38 KPC R′ 5′-TAACTTACAGTTGCGCCTGAG-3′ SEQ ID NO. 39 KPC-FAM 5′-/5TYE665/ATCGGTGTGTACGCGATGGATACC/3BHQ_2/-3′ SEQ ID NO. 40 VIM F′ 5′-CATTCGACCGACAACTTAG-3′ SEQ ID NO. 41 VIM R′ 5′-CGTGCGTGACAACTCAT-3′ SEQ ID NO. 42 VIM-TEX 5′45TEX615/TGTGCTCTATGGTGGTTGTGCGAT/3BHQ_2/-3′ SEQ ID NO. 43 DHA F′ 5′-TGCGTACGGTTATGAGAACAA-3′ SEQ ID NO. 44 DHA R′ 5′-CCCAGCGCAGCATATCTT-3′ SEQ ID NO. 45 DHA-FAM 5′-/56-FAM/ATGCGGAAT/ZEN/CTTACGGCGTGAAAT/3IABkFQ-3′ SEQ ID NO. 46 IMP F′

5′-ACGTAGTGGTTTGGTTACCTG-3′ SEQ ID NO. 47 IMP R′ 5′-
AAGCTTCTAAATTTGCGTCACC-3′ SEQ ID NO. 48 IMP-
5′-/5HEX/TTTGTTAAA/ZEN/CCGGACGGTCTTGGT/3IABkFQ/-3′ TYE705
(128) TABLE-US-00004 TABLE 3 DNA Control Sequence SEQ ID NO. 49 MOX
AACCGGGAGAGCGGGGCCAGCGTCAGCGAGCAGACCCTGTTCGAGATAG
GATCCGTGAGCAAGACCCTGACTGCGACCCTGGGGGCCTATGCGGTGGTC
AAGGGAGCGATGCAGCTGGATGACAAGGCGAGCCGGCACGCGCCCTGGC
TCAAGGGATCCGTCTTTGACAGCATCACCATGGGGGAGCTTGCCACCTAC AGC SEQ
ID NO. 50 FOX
GGGGATGGCGGTCGCCGTGCTGAAAGATGGCAAGGCCCACTATTTCAACT
ATGGGGTTGCCAACCGCGAGAGTGGTCAGCGCGTCAGCGAGCAGACCCT
GTTCGAGATTGGCTCGGTCAGCAAGACCCTGACCGCGACCCTCGGTGCCT
ATGCTGCGGTCAAGGGGGGCTTTGAGCTGGATGACAAGGTGAGCCAGCA
CGCCCCCTGGCTCAAAGGTTCCGCCTTTGATGGTGTGACCAT SEQ ID NO. 51 EBC
GGACCGTTACGCCGCTGATGAAAGCGCAGGCCATTCCGGGTATGGCGGT
GGCGGTGATTTATGAGGGTCAGCCGCACTACTTCACCTTCGGTAAAGCCG
ATGTTGCGGCGAACAAACCTGTCACTCCACAAACCTTGTTCGAACTGGGTT
CTATAAGTAAAACCTTCACCGGCGTACTCGGTGGCGATGCCATTGCTCGCG
GTGAAATATCGCTGGGCGA SEQ ID NO. 52 DHA
GACTGCACGGATCCTGGCACCGCTGGGGTTATCTCACACCTTTATTACTGT
GCCGGAAAGTGCGCAAAGCCAGTATGCGTACGGTTATGAGAACAAAAAA
CCGGTCCGCGTGTCGCCGGGACAGCTTGATGCGGAATCTTACGGCGTGGA
ATCCGCCTCAAAAGATATGCTGCGCTGGGCGGAAATGAATATGGAGCCGT
CACGGGCCGGTAATGCGGAT SEQ ID NO. 53 CMY
GCCTGTACACGTTTCTCCGGGACAACTTGACGCCGAAGCCTATGGCGTGA
AATCCAGCGTTATTGATATGGCCCGCTGGGTTCAGGTCAACATGGACGCC
AGCCGCGTTCAGGAGAAAACGCTCCAGCAGGGCATTGCGCTTGCGCAGTC
TCGCTACTGGCGTATTGGCGATATGTACCAGGGATTAGGCTGGGAGATGC
TGAACTGGCCGCTGAAAGCTGATTCGATCATCAACGGTAGCGACAGCAAA
GTGGCATTGG SEQ ID NO. 54 ACC
GAGAGCAAAATTAAAGACACCGTTGATGACCTGATCCAGCCGCTGATGCA
GAAGAATAATATTCCCGGTATGTCGGTCGCAGTGACCGTCAACGGTAAAA
ACTACATTTATAACTATGGGTTAGCGGCAAAACAGCCTCAGCAGCCGGTT SEQ ID
NO. 55 IC AGCTTGTTGGTGGGGTAACGGCTCACCAAGGCGACGATCCCTAGCTGGTC
TGAGAGGATGACCAGCCACACTGGAACTGAGACACGGTCCAGACTCCTAC
GGGAGGCAGCAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAG
CCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAAGTACTTTCAGCG
GGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCAG
AAGAAGCACCGGCTAACTCCG SEQ ID NO. 56 CTX-M-
CGTTTCGTCTGGATCGCACTGAACCTACGCTGAATACCGCCATTCCCGGCG 14
ACCCGAGAGACACCACCACGCCGCGGGCGATGGCGCAGACGTTGCGTCA
GCTTACGCTGGGTCATGCGCTGGGCGAAACCCAGCGGGCGCAGTTGGTG
ACGTGGCTCAAAGGCAATACGACCGGCGCAGCCAGCATTCGGGCCGGCTT
ACCGACGTCGTGGACTGTGGGTGATAAGACCGGCAGCGGCGACTACGGC
ACCACCAATGATATTGCGGTGATCTGGCCGCAGGGTCGTGCGCCGCTGGT
TCTGGTGACCTATTTTACCCAGC SEQ ID NO. 57 CTX-M-
CCGTCACGCTGTTGTTAGGAAGTGTGCCGCTGTATGCGCAAACGGCGGAC 15
GTACAGCAAAAACTTGCCGAATTAGAGCGGCAGTCGGGAGGCAGACTGG
GTGTGGCATTGATTAACACAGC SEQ ID NO. 58 OXA
AATCACAGGGCGTAGTTGTGCTCTGGAATGAGAATAAGCAGCAAGGATTT

ACCAATAATCTTAAACGGGCGAACCAAGCATTTTTACCCGCATCTACCTTTA
AAATTCCCAATAGCTTGATCGCCCTCGATTTGGGCGTGGTTAAGGATGAAC
ACCAAGTCTTTAAGTGGGATGGACAGACGCGCGATATCGCCACTTGGAAT
CGCGATCATAATCTAATCACCGCGATGAAATATTCAGTTGTGCCTGTTTAT
CAAGAATTTGCCCGCCAAATTGGCGAGGCACGTATGAGCAAGATGCTACA
TGCTTTCGATTATGGTAATGAGGACATTTCGGGCAATGTAGACAGTTTCTG
GCTCGACGGTGGTATTCGAATTTCGGCCACGGAGCAAATCAGCTTTTTAA
GAAAGCTGTATCACAATAAGTTACACGTATCGGAGCGCAGCCAGCGTATT
GTCAAACAAGCCATGCTGACCGAAGCCAATGGTGACTAATTATTCGGGCT
AAAACTGGATACTCGACTAGAATCGAACCTAAGATTGGCTGGCTGGGT SEQ ID
NO. 59 IC CGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATGAGCAAAGGTAT
TAACTTTACTCCCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTT
CTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATT
CCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTG
GCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTTGGTGAGCCGTTA
CCCCACCAACAAGCT SEQ ID NO. 60 CMY
GCCTGTACACGTTTCTCCGGGACAACTTGACGCCGAAGCCTATGGCGTGA
AATCCAGCGTTATTGATATGGCCCGCTGGGTTCAGGTCAACATGGACGCC
AGCCGCGTTCAGGAGAAAACGCTCCAGCAGGGCATTGCGCTTGCGCAGTC
TCGCTACTGGCGTATTGGCGATATGTACCAGGGATTAGGCTGGGAGATGC
TGAACTGGCCGCTGAAAGCTGATTCGATCATCAACGGTAGCGACAGCAAA
GTGGCATTGG SEQ ID NO. 61 NDM
GGCGAAAGTCAGGCTGTGTTGCGCCGCAACCATCCCCTCTTGCGGGGCAA
GCTGGTTCGACAACGCATTGGCATAAGTCGCAATCCCCGCCGCATGCAGC
GCGTCCATACCGCCCATCTTGTCCTGATGCGCGTGAGTCACCACCGCCAGC
GCGACCGGCAGGTTGATCTCCTGCTTGATCCAGTTGAGGATCTGGGCGGT
CTGGTCATCGGTCCAGGCGGTATCGACCACCAGCACGCGGCCGCCATCCC
TGACGATCAAAC SEQ ID NO. 62 KPC
GTATCGCCGTCTAGTTCTGCTGTCTTGTCTCTCATGGCCGCTGGCTGGCTTT
TCTGCCACCGCGCTGACCAACCTCGTCGCGGAACCATTCGCTAAACTCGAA
CAGGACTTTGGCGGCTCCATCGGTGTGTACGCGATGGATACCGGCTCAGG
CGCAACTGTAAGTTACCGCGCTGAGGAGCGCTTCCCACTGTGCAGCTCATT CAAGG
SEQ ID NO. 63 VIM
CCATTCAGCCAGATCGGCATCGGCCACGTTCCCCGCAGACGTGCGTGACA
ACTCATGAATCGCACAACCACCATAGAGCACACTCGCAGACGGGACGTAC
ACAACTAAGTTGTCGGTCGAATGCGCAGCACCAGGATAGAAGAGTTCTAC
TGGACCGAAGCGCACTGCGTCCCCGCTCGAGTCCTTCTAGAGAGTGCGTG
GGAATCTCGTTCCCCTCTACCTCGGCTAGCCGGCGTGTCGACGGTGATGC
GTACGTTGCCACCCCAGCCGCCCGAAGGACATCAACGCCGCC SEQ ID NO. 64 DHA
GACTGCACGGATCCTGGCACCGCTGGGGTTATCTCACACCTTTATTACTGT
GCCGGAAAGTGCGCAAAGCCAGTATGCGTACGGTTATGAGAACAAAAAA
CCGGTCCGCGTGTCGCCGGGACAGCTTGATGCGGAATCTTACGGCGTGAA
ATCCGCCTCAAAAGATATGCTGCGCTGGGCGGAAATGAATATGGAGCCGT
CACGGGCCGGTAATGCGGAT SEQ ID NO. 65 IC
CGGAGTTAGCCGGTGCTTCTTCTGCGGGTAACGTCAATGAGCAAAGGTAT
TAACTTTACTCCCTTCCTCCCCGCTGAAAGTACTTTACAACCCGAAGGCCTT
CTTCATACACGCGGCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATT
CCCCACTGCTGCCTCCCGTAGGAGTCTGGACCGTGTCTCAGTTCCAGTGTG
GCTGGTCATCCTCTCAGACCAGCTAGGGATCGTCGCCTTGGTGAGCCGTTA
CCCCACCAACAAGCT SEQ ID NO. 66 IMP

GCGGAGTTAGTTATTGGCTAGTTAAAAATAAAATTGAAGTTTTTTATCCCG
GCCCGGGGCACACTCAAGATAACGTAGTGGTTTGGTTACCTGAAAAGAAA
ATTTTATTCGGTGGTTGTTTTGTTAAACCGGACGGTCTTGGTAATTTGGGT
GACGCAAATTTAGAAGCTTGGCC

(129) The sequence listing including SEQ ID NOS 1-295 is hereby incorporated by reference for all purposes.

(130) Unless otherwise stated, any numerical values recited herein include all values from the lower value to the upper value in increments of one unit provided that there is a separation of at least 2 units between any lower value and any higher value. As an example, if it is stated that the amount of a component, a property, or a value of a process variable such as, for example, temperature, pressure, time and the like is, for example, from 1 to 90, preferably from 20 to 80, more preferably from 30 to 70, it is intended that intermediate range values such as (for example, 15 to 85, 22 to 68, 43 to 51, 30 to 32 etc.) are within the teachings of this specification. Likewise, individual intermediate values are also within the present teachings. For values which are less than one, one unit is considered to be 0.0001, 0.001, 0.01, or 0.1 as appropriate. These are only examples of what is specifically intended and all possible combinations of numerical values between the lowest value and the highest value enumerated are to be considered to be expressly stated in this application in a similar manner. As can be seen, the teaching of amounts expressed as "parts by weight" herein also contemplates the same ranges expressed in terms of percent by weight. Thus, an expression in the of a range in terms of "at least 'x' parts by weight of the resulting composition" also contemplates a teaching of ranges of same recited amount of "x" in percent by weight of the resulting composition."

(131) Unless otherwise stated, all ranges include both endpoints and all numbers between the endpoints. The use of "about" or "approximately" in connection with a range applies to both ends of the range. Thus, "about 20 to 30" is intended to cover "about 20 to about 30", inclusive of at least the specified endpoints.

(132) The disclosures of all articles and references, including patent applications and publications, are incorporated by reference for ail purposes. The term "consisting essentially of to describe a combination shall include the elements, ingredients, components or steps identified, and such other elements ingredients, components or steps that do not materially affect the basic and novel characteristics of the combination. The use of the terms "comprising" or "including" to describe combinations of elements, ingredients, components or steps herein also contemplates embodiments that consist of, or consist essentially of the elements, ingredients, components or steps.

(133) Plural elements, ingredients, components or steps can be provided by a single integrated element, ingredient, component or step. Alternatively, a single integrated element, ingredient, component or step might be divided into separate plural elements, ingredients, components or steps. The disclosure of "a" or "one" to describe an element, ingredient, component or step is not intended to foreclose additional elements, ingredients, components or steps.

(134) It is understood that the above description is intended to be illustrative and not restrictive. Many embodiments as well as many applications besides the examples provided will be apparent to those of skill in the art upon reading the above description. The scope of the invention should, therefore, be determined not with reference to the above description, but should instead be determined with reference to the appended claims, along with the full scope of equivalents to which such claims are entitled. The disclosures of all articles and references, including patent applications and publications, are incorporated by reference for all purposes. The omission in the following claims of any aspect of subject matter that is disclosed herein is not a disclaimer of such subject matter, nor should it be regarded that the inventors did not consider such subject matter to be part of the disclosed inventive subject matter.

Claims

1. A kit for identification of one or more β-lactamase genes, wherein the one or more β-lactamase genes are selected from the group consisting of: CMY, CTX-M, OXA, IMP, VIM, DHA, KPC, and NDM genes, the kit comprising probes comprising the following sequences:
ACGCTAACTCCAGCATTGGTCTGT (SEQ ID NO: 154),
AACTTGCCGAATTAGAGCRGCAGT (SEQ ID NO: 157),
TATCATTGGTGGTGCCGTAGTCGC (SEQ ID NO: 160),
TGAGACACGGTCCAGACTCCTACG (SEQ ID NO: 163),
TAGCTTGATCGCCCTCGATTTGGG (SEQ ID NO: 166), TTRTTYGGTGGTTGYTTTRTTAA (SEQ ID NO: 169), CCGGACGGTCTTGGTAATTTGGGT (SEQ ID NO: 172), CCGTACGGTTTAGGCAATTTGGGT (SEQ ID NO: 173), AGCTCTTCTATCCTGGTGCTGCG (SEQ ID NO: 176), TGAGACACGGTCCAGACTCCTACG (SEQ ID NO: 179), AAACCGGGCGATATGCGTCTGTAT (SEQ ID NO: 182), TCGTCGCGGAACCATTCGCTAAA (SEQ ID NO: 185), CATCAGGACAAGATGGGCGGTATG (SEQ ID NO: 188), and TGAGACACGGTCCAGACTCCTACG (SEQ ID NO: 191), wherein each probe comprises a fluorophore and/or a fluorescent quencher.
2. The kit of claim 1, including an endogenous internal control.
3. The kit of claim 2, wherein the endogenous internal control targets a conserved region in Gram-negative bacteria.
4. The kit of claim 1, further comprising the following primers:
TGGCCAGAACTGACAGGCAAA (SEQ ID NO: 152), TTTCTCCTGAACGTGGCTGGC (SEQ ID NO: 153), CCGTCACGCTGTTGTTAGG (SEQ ID NO: 155),
GCTGTGTTAATCAATGCCACAC (SEQ ID NO: 156), CGTTTCGTCTGGATCGCAC (SEQ ID NO: 158), GCTGGGTAAAATAGGTCACC (SEQ ID NO: 159),
GAGAGGATGAYCAGCCACAC (SEQ ID NO: 161), and CGCCCATTGTSCAATATTCC (SEQ ID NO: 162).
5. The kit of claim 4, further comprising the following primers: AATCACAGGGCGTAGTTGTG (SEQ ID NO: 164), ACCCACCAGCCAATCTTAGG (SEQ ID NO: 165),
GCGGAGTTAACTATTGGCTAG (SEQ ID NO: 167), GGCCAAGCTTCTATATTTGCG (SEQ ID NO: 168), GCGGAGTTARYTATTGGCTAG (SEQ ID NO: 170),
GGCCAAGCYTCTAWATTTGCG (SEQ ID NO: 171), GGCGGCGTTGATGTCCTTCG (SEQ ID NO: 174), CCATTCAGCCAGATCGGCATC (SEQ ID NO: 175),
GAGAGGATGAYCAGCCACAC (SEQ ID NO: 177), and CGCCCATTGTSCAATATTCC (SEQ ID NO: 178).
6. The kit of claim 5, further comprising the following primers:
AACTTTCACAGGTGTGCTGGGT (SEQ ID NO: 180), CCGTACGCATACTGGCTTTGC (SEQ ID NO: 181), GTATCGCCGTCTAGTTCTGC (SEQ ID NO: 183),
CCTTGAATGAGCTGCACAGTGG (SEQ ID NO: 184), GTTTGATCGTCAGGGATGGC (SEQ ID NO: 186), GGCGAAAGTCAGGCTGTG (SEQ ID NO: 187),
GAGAGGATGAYCAGCCACAC (SEQ ID NO: 189), and CGCCCATTGTSCAATATTCC (SEQ ID NO: 190).
7. The kit of claim 1, including at least one control DNA mix.
8. The kit of claim 1, including exactly two control DNA mixes.
9. The kit of claim 1, including exactly three control DNA mixes.
10. The kit of claim 1, including a composition containing a tracking dye.