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(72) Inventors; and
(71) Applicants : **BOGOCH, Samuel** [CA/US]; 46 E. 91st Street, New York, NY 10128 (US). **BOROCH, Elenore, S.** [US/US]; 46 E. 91st Street, New York, NY 10128 (US). **BOGOCH, Samuel, Winston** [CA/US]; 429 - 62nd Street, Oakland, CA 94609 (US). **BORSANYI, Anne-elenore, Bogoch** [CA/US]; 46 E. 91st Street, New York, NY 10128 (US).

(74) Agent: **NICHOLSON, Daren, P.**; 5065 Cainsville Road, Lebanon, TN 37090 (US).

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(54) Title: METHODS OF DIAGNOSING, PREVENTING, AND TREATING DRUG-RESISTANT PATHOGENIC INFECTIONS USING REPLIKIN SEQUENCES

(57) Abstract: The present invention provides methods of determining if a pathogen or population of pathogen is drug sensitive or drug resistant and compounds and compositions for prevention and treatment of infections of drug-resistant pathogens.

METHODS OF DIAGNOSING, PREVENTING, AND TREATING DRUG-RESISTANT PATHOGENIC INFECTIONS USING REPLIKIN SEQUENCES

[0001] This application claims priority to U.S. Provisional Appln. Ser. No. 61/779,324, filed March 13, 2013.

[0002] This application incorporates by reference in its entirety each of the following applications: PCT/US2014/016303, filed February 13, 2014, PCT/US2013/0069310, filed November 8, 2013, PCT/US2013/039111, filed May 1, 2013, U.S. Appln. Ser. No. 13/791,609, filed March 8, 2013, PCT/US2013/030013, filed March 8, 2013, U.S. Provisional Appln. Ser. No. 61/765,106, filed February 15, 2013, U.S. Provisional Appln. Ser. No. 61/724,538, filed November 9, 2012, U.S. Appln. Ser. No. 13/553,137, filed July 19, 2012, PCT/US2012/047451, filed July 19, 2012, U.S. Provisional Appln. Ser. No. 61/609,074, filed March 9, 2012, U.S. Appln. Ser. No. 12/581,112, filed October 16, 2009, U.S. Provisional Appln. Ser. No. 61/246,006, filed September 25, 2009, U.S. Appln. Ser. No. 12/538,027, filed August 7, 2009, U.S. Provisional Appln. Ser. No. 61/185,160, filed June 8, 2009, U.S. Provisional Appln. Ser. No. 61/179,686, filed May 19, 2009, U.S. Provisional Appln. Ser. No. 61/172,115, filed April 23, 2009, U.S. Appln. Ser. No. 12/429,044, filed April 23, 2009, PCT/US09/41565, filed April 23, 2009, and U.S. Provisional Appln. Ser. No. 61/143,618, filed January 9, 2009, U.S. Provisional Appln. Ser. No. 61/087,354, filed August 8, 2008, U.S. Provisional Appln. Ser. No. 61/054,010, filed May 16, 2008, U.S. Appln. Ser. No. 12/108,458, filed April 23, 2008, PCT/US2008/61336, filed April 23, 2008, U.S. Appln. Ser. No. 12/010,027, filed January 18, 2008, U.S. Provisional Appln. Ser. No. 60/991,676, filed November 30, 2007, U.S. Appln. Ser. No. 11/923,559, filed October 24, 2007, now U.S. Patent No. 8,050,871, U.S. Provisional Appln. Ser. No. 60/982,336, filed October 24, 2007, U.S. Provisional Appln. Ser. No. 60/982,333, filed October 24, 2007, U.S. Provisional Appln. Ser. No. 60/982,338, filed October 24, 2007, U.S. Provisional Appln. Ser. No. 60/935,816, filed August 31, 2007, U.S. Provisional Appln. Ser. No. 60/935,499 filed August 16, 2007, U.S. Provisional Appln. Ser. No. 60/954,743, filed August 8, 2007, U.S. Appln. Ser. No. 11/755,597, filed May 30, 2007, U.S. Provisional Appln. Ser. No. 60/898,097, filed January 30, 2007, U.S. Provisional Appln. Ser. No. 60/880,966, filed January 18, 2007, U.S. Provisional Appln. Ser. No. 60/853,744, filed October 24, 2006, U.S. Appln. Ser. No. 11/355,120, filed February 16, 2006, now U.S. Patent No. 7,894,999, U.S. Appln. Ser. No. 11/116,203, filed April 28, 2005, now U.S. Patent No. 7,774,144, U.S. Appln. Ser. No. 10/860,050, filed June 4, 2004, now U.S. Patent No. 7,442,761, U.S. Appln. Ser. No.

10/189,437, filed July 8, 2002, now U.S. Patent No. 7,452,963, U.S. Appln. Ser. No. 10/105,232, filed March 26, 2002, now U.S. Patent No. 7,189,800, U.S. Appln. Ser. No. 09/984,057, filed October 26, 2001, now U.S. Patent No. 7,420,028, and U.S. Appln. Ser. No. 09/984,056, filed October 26, 2001, now U.S. Patent No. 7,176,275.

SEQUENCE LISTING

[0002.1] The instant application contains a Sequence Listing, which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 11, 2014, is named 13795-48676_SL.txt and is 20,245 bytes in size.

BACKGROUND OF THE INVENTION

[0003] Antimicrobial agents have been in use in modern medicine for eight decades against pathogenic infections and have greatly reduced morbidity and mortality from infectious diseases. Nevertheless, because of their wide-spread use and effectiveness over time (both in animals and humans), certain pathogenic microorganisms have developed resistance, rendering some antimicrobial therapies less effective or even ineffective against pathogens previously susceptible to the drugs. Resistance to antimicrobials has been identified in fungi, viruses, parasites, and bacteria. Certain pathogens have developed resistance to one antimicrobial therapy while other pathogens have developed resistance to more than one therapy including more than one class of antibiotic therapy. In certain instances, resistance has evolved such that no known antimicrobial agent against a specific pathogen is available for effective treatment. Drug-resistance in these pathogens is tragic and expensive with more death, longer hospitalization, and more suffering. Additionally, drugs that are used to replace ineffective therapies may be less effective, more expensive, and/or more toxic.

[0004] Pubic health officials in the U.S. and globally have expressed alarm at the rise of antimicrobial resistance in pathogens. The chief medical officer of the United Kingdom has noted that antimicrobial resistance reflects a catastrophic threat to public health and asserted that the global health community is currently in a race against time to develop therapies for drug-resistant microbes. The officer called for more research into new antibiotics combined with better surveillance, including more genomic testing to increase scientists' understanding of emerging resistance. This alert comes amid rising international concern about drug

resistance. Nature News Blog (11 Mar 2013) (http://blogs.nature.com/news/2013/03/drug-resistant-bacteria-and-lack-of-new-antibiotics-pose-catastrophic-threat.html).

[0005] Resistance to antibiotic treatment is a particularly-relevant form of drug resistance wherein sub-populations of a pathogen survive exposure to one or more antibiotic therapies resulting in a sub-population that is not treatable with previously-effective antibiotic therapies or previously-effective doses of antibiotic therapies. Development of resistance in certain sub-populations is a significant threat in public health including a significant threat in nosocomial diseases. Certain nosocomial pathogens have developed resistance to multiple antibiotic families. These populations are considered multidrug resistant pathogens.

[0006] Drug-resistant pathogens include, for example, Extensively Drug-resistant Tuberculosis, Gonococcus, Klebsiella, Clostridium, Streptococcus, Methicillin-resistant Staphylococcus, and drug-resistant E. coli. Resistance has also been noted in some fungi, including in patients with weakened immune systems. Fungi with populations demonstrating antimicrobial resistance include *Candida albicans, Candida glabrata,* and Aspergillus spp. Antimicrobial resistance in malaria has been a continuing problem for many years. Antimicrobial resistance has likewise been observed in a wide-range of other parasites.

[0007] Tuberculosis remains a major infectious disease, accounting for approximately 3 million deaths per year worldwide. A series of effective drugs were developed over several decades that have recently been challenged by two new circumstances. The first challenging circumstance is the mutation of the tuberculosis genome to more lethal drug resistant forms. The second challenging circumstance is the association of tuberculosis with AIDS, both resulting in high mortality cases in such areas as KwaZulu-Natal in South Africa and in Europe and Asia. Extensively Drug-Resistant Tuberculosis has previously been defined as tuberculosis that demonstrates resistance to at least isoniazid and rifampin, resistance to any member of the fluoroquinolone family, and resistance to at least one second-line drug, which would include, for example, kanamycin, amikacin, and capreomycin.

[0008] Drug-resistant Gonococcus populations have been identified as resistant to penicillin, tetracycline, quinolone, rifampicin, and cefixime. In addition, a high-level ceftriaxone-resistant strain has now been identified.

[0009] Klebsiella populations expressing carbapenase enzyme have likewise been identified with resistance to the class of carbapenem antibiotic therapies. Multi-drug resistant populations have additionally been identified.

[00010] A strain of *Clostridium difficile* has also now been identified that is resistant to flouroquinolone antibiotics. This strain is highly-toxic and is resistant to antibiotics such as ciprofloxacin and levofloxacin. Since 2005, the strain has been reported to cause geographically-dispersed outbreaks and epidemics.

[00011] Populations of methicillin-resistant staphylococcus aureus (MRSA) also continue to be a threat, particularly in hospital (nosocomial) settings. MRSA infections may result in pneumonia or skin and tissue necrosis in healthy people. Certain populations of MRSA have been reported to be resistant to most-commonly prescribed medications but are considered to be generally susceptible to Vancomycin therapy. Nevertheless, three classes of Vancomycin-resistant *Staphylococcus aureus* have emerged following standard treatment of MRSA with Vancomycin.

[00012] Streptococcus species are another family of bacteria observed to be resistant to common drug therapies such as penicillin.

[00013] Multi-drug resistant strains of *Escherichia coli* have also been identified.

[00014] Vaccines against these drug-resistant pathogens have been considered. No satisfactory vaccine has yet to be produced. In some circumstance, vaccine development has been hindered by limited acquired immunity to infection. The present invention, however, provides surprising therapies and vaccines for drug-resistant pathogens.

[00015] The inventors have previously identified a family of conserved, small protein sequences that relate to rapid replication in influenza and other viruses. The family of protein sequences is known as Replikins. Rapid replication is characteristic of virulence in viruses where Replikin sequences are identified or where concentrations of Replikin sequences are present. Rapid replication has been associated with the presence of Replikin sequence structures in protein sequences. Replikin sequences have been further associated with viral outbreaks, epidemics and increased rates of host mortality.

[00016] Replikin sequences were first discovered in glioblastoma multiforme brain cancer cells rapidly replicating in tissue culture and have since been additionally identified as virus structures where increasing concentration is correlated quantitatively with strain-specific virus outbreaks as well as initial geographic location of such outbreaks.

[00017] Identification of Replikin peptides has provided targets for detection and treatment of pathogens, including vaccine development against virulent pathogens such as influenza virus, malaria, West Nile virus, and foot and mouth disease virus. *See, e.g.,* WO 2008/143717. In general, knowledge of and identification of this family of peptides enables

development of effective therapies and vaccines for pathogens that harbor Replikins. The phenomenon of the association of Replikins with rapid replication and virulence has been fully described in U.S. Patent No. 7,189,800; U.S. Patent No. 7,176,275; U.S. Patent No. 7,442,761; U.S. Patent No. 7,894,999, U.S. Patent No. 8,050,871, and U.S. Appln. Ser. No. 12/108,458. Both Replikin concentration (number of Replikins per 100 amino acids) and Replikin composition have been correlated with the functional phenomenon of rapid replication.

[00018] The area of a genome of the highest concentration of Replikin sequences in a virus such as influenza virus, and the country in which this peak high concentration exists in scout viruses, has permitted in the past five years twelve consecutive accurate predictions of the geographic localization of coming outbreaks of influenza. These predictions include those now realized in Mexico for H1N1, in Cambodia for H5N1, and in the United States for H3N2. Real-time Replikin analysis of the evolution of the influenza virus genome identified both mutations and structural reorganization of the hemagglutinin and pB1 genes over several years before each predicted outbreaks. This information, together with the specific Replikin sequences so obtained, permitted solid-phase synthesis of Replikin vaccines in seven days, which blocked Taura Syndrome Virus in shrimp and blocked H5N1 influenza virus in chickens. *See, e.g.,* WO 2008/156914, Figures 1 and 2 and WO 2010/123519, Figure 1.

[00019] Prior to the present invention, Replikin sequences had not been associated with drug resistance in pathogens. Further, no structures of any kind in infectious organisms had been described that correlate quantitatively and temporally with increases in drug resistance in pathogens or with outbreaks of drug-resistant pathogens. No structures had been described that correlate with course of outbreak and lethality of infections and no structure had been identified that permits early or advance warning of such outbreaks.

[00020] The art is currently in need of methods of diagnosing and identifying drug-resistant pathogens and the rise of drug-resistant pathogens temporally or in specific geographic locations, such as countries or clinical settings. The art is further currently in need of therapies against such drug-resistant pathogens including vaccines against such drug-resistant pathogens.

SUMMARY OF THE INVENTION

[00021] The present invention provides methods of determining if a population of pathogen is drug sensitive or drug resistant and compounds and compositions for prevention and treatment of infections of drug resistant pathogens.

[00022] A first non-limiting aspect of the present invention provides a method of determining if a pathogen or population of pathogen is drug sensitive or drug resistant. A non-limiting embodiment of the first aspect of the present invention provides a method of diagnosing the drug sensitivity or drug resistance of a pathogen comprising determining a Replikin concentration of said pathogen and diagnosing the pathogen as drug resistant if the Replikin concentration is greater than 4.0 per 100 amino acid residues and drug sensitive if the Replikin concentration is 4.0 or less. In a non-limiting embodiment, the pathogen is a bacterial pathogen. In another non-limiting embodiment, the bacterial pathogen is a Gonococcus pathogen, a Tuberculosis pathogen, a Klebsiella pathogen, a Clostridium pathogen, a Streptococcus pathogen, a Staphylococcus pathogen, or an E. coli pathogen. In a non-limiting embodiment, the pathogen is *Neisseria gonorrhoeae*, *Mycobacterium tuberculosis*, *Klebsiella pneumoniae, Clostridium difficile,* or *Streptococcus pneumoniae*. In a non-limiting embodiment, the pathogen is a fungus. In another non-limiting embodiment, the pathogen is *Candida albicans, Candida glabrata,* or Aspergillus. In another non-limiting embodiment, the pathogen is malaria or other parasitic pathogen.

[00023] A non-limiting embodiment of the first aspect of the invention provides a method of diagnosing if a population of pathogens is increasing in drug resistance comprising (1) determining a Replikin concentration of a plurality of specimens of an initial population of pathogen at a given time or in a given location and determining the percentage of said specimens having a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues, (2) determining a Replikin concentration of a plurality of specimens of a different population of the pathogen at a different time or different location and determining the percentage of said specimens having a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues, and (3) if the percentage of specimens having a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues in the initial population of pathogens is greater than the percentage of specimens having a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues in the different population, diagnosing the initial population of pathogens as increasing in drug resistance. In a non-limiting embodiment, the population of pathogens is a population of

bacterial pathogen. In another non-limiting embodiment, the bacterial pathogen is a Gonococcus pathogen, a Tuberculosis pathogen, a Klebsiella pathogen, a Clostridium pathogen, a Streptococcus pathogen, a Staphylococcus pathogen, or an E. coli pathogen. In a non-limiting embodiment, the pathogen is *Neisseria gonorrhoeae*, *Mycobacterium tuberculosis*, *Klebsiella pneumoniae, Clostridium difficile,* or *Streptococcus pneumoniae*. In a non-limiting embodiment, the pathogen is a fungus. In another non-limiting embodiment, the pathogen is *Candida albicans, Candida glabrata,* or Aspergillus. In another non-limiting embodiment, the pathogen is malaria or other parasitic pathogen.

[00024] A non-limiting embodiment of the first aspect of the invention provides a method of determining an increase in drug-resistance in a population of a pathogen, comprising determining the percent of isolates of the pathogen from a population at a first and a second time period having a Replikin concentration of greater than 4.0 and if the percent of isolates having a Replikin concentration of greater than 4.0 increases between the first and second time period, determining that the pathogenic population is increasing in drug resistance from the first to the second time period.

[00025] A further non-limiting embodiment of the first aspect of the invention provides a computer readable medium having stored thereon instructions, which when executed, cause a processor to perform a method of determining if a pathogen or population of pathogen is drug sensitive or drug resistant. In a further embodiment, the processor reports a prediction to a display, user, researcher, or other machine or person. In a further embodiment, the processor identifies to a display, user, researcher, or other machine or person, a portion of a pathogen predicted to be drug-resistant, wherein said portion may be employed as a therapeutic or diagnostic compound. Said portion may be a Replikin peptide sequence or plurality of Replikin peptide sequences or any other structure or portion of said genome of said pathogen including a Replikin Peak Gene. A non-limiting computer readable medium may be non-transitory. Software comprising methods of the invention and related data may be carried on a signal.

[00026] A second non-limiting aspect of the present invention provides an isolated or synthesized protein fragment or peptide comprising at least one peptide sequence that is at least 50% homologous with at least one Replikin peptide sequence identified in a pathogen identified in a population of pathogens having a higher percentage of specimens with a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues than specimens of another population of the same pathogen. In a non-limiting embodiment,

the at least one peptide sequence may be at least 80% homologous with at least one Replikin peptide sequence so identified. In a non-limiting embodiment, the at least one peptide sequence may be at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% or more homologous with at least one Replikin peptide so identified. In another non-limiting embodiment, the at least one peptide sequence may be at least 53%, 57%, 64%, 67%, 70%, 76%, 80%, 82%, 87%, 91%, or 93% or more homologous with at least one Replikin peptide so identified. In a non-limiting embodiment, the population of pathogen is a bacterial pathogen, fungal pathogen, or parasitic pathogen. In another non-limiting embodiment, the bacterial pathogen is a Gonococcus pathogen, Tuberculosis pathogen, Klebsiella pathogen, Clostridium pathogen, Streptococcus pathogen, Staphylococcus pathogen, or E. coli pathogen. In another non-limiting embodiment, the bacterial pathogen is *Neisseria gonorrhoeae*, *Mycobacterium tuberculosis*, *Klebsiella pneumoniae, Clostridium difficile,* or *Streptococcus pneumoniae*. In a non-limiting embodiment, the pathogen is a fungus. The fungus may include, but is not limited to, *Candida albicans, Candida glabrata,* and Aspergillus spp. The pathogen may also be a malarial pathogen or other parasitic pathogen.

[00027] In another non-limiting embodiment, the isolated or synthesized protein fragment or peptide may comprise at least one Replikin peptide sequence or at least one homologue of the at least one Replikin sequence. The isolated or synthesized protein fragment or peptide may consist essentially of at least one Replikin peptide or at least one homologue of said at least one Replikin peptide sequence. The isolated or synthesized protein fragment or peptide may consist of at least one Replikin peptide sequence or at least one homologue of said at least one Replikin peptide sequence. Another non-limiting embodiment provides an isolated or synthesized peptide sequence comprising at least one functional fragment of a Replikin sequence identified in a population of pathogens having a higher percentage of specimens with a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues than specimens of another population of the same pathogen.

[00028] In a non-limiting embodiment, the isolated or synthesized protein fragment or peptide may comprise at least one Replikin peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119 or at least one homologue of SEQ ID NO(s): 1-21, 22-56, or 57-119. In a non-limiting embodiment, the isolated or synthesized protein fragment or peptide may consists essentially of at least one Replikin peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119 or at least one homologue of SEQ ID NO(s): 1-21, 22-56, or 57-119. In another non-limiting embodiment, the isolated or synthesized protein fragment or peptide may consist of

at least one Replikin peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119 or at least one homologue of SEQ ID NO(s): 1-21, 22-56, or 57-119. Another non-limiting embodiment provides an isolated or synthesized peptide sequence comprising at least one functional fragment of at least one Replikin peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119.

[00029] In a non-limiting embodiment of the second aspect of the invention, a homologue and a functional fragment of a Replikin sequence includes and is not limited to SEQ ID NO(s): 120-122 and homologues and functional fragments likewise understood by the ordinary skilled artisan upon reviewing the teachings of this specification.

[00030] A non-limiting embodiment of the second aspect of the invention provides an isolated or synthesized protein, protein fragment, polypeptide, or peptide comprising at least one Replikin peptide of a drug-resistant pathogen. In a further embodiment, the drug-resistant pathogen is a drug-resistant bacterial pathogen. A further embodiment of the second aspect of the invention provides an isolated or synthesized protein, protein fragment, polypeptide, or peptide comprising at least one peptide sequence that is at least 30%, 40%, 50%, 60%, 70%, 80%, 90% 95%, or 100%, homologous with at least one Replikin peptide sequence identified in a drug-resistant pathogen. In a non-limiting embodiment, the at least one sequence is one of SEQ ID NO(s): 1-21, 22-56, or 57-119.

[00031] In a non-limiting embodiment, an isolated or synthesized protein fragment or peptide may consist of 50, 60, 70, 80, 90, 100, 150, or up to 200 amino acid residues. The isolated or synthesized protein fragment or peptide comprises at least one Replikin peptide sequence, at least one homologue of at least one Replikin sequence, or at least one functional fragment of at least one Replikin sequence.

[00032] In a further non-limiting embodiment of the second aspect of the present invention, the isolated or synthesized protein, protein fragment, polypeptide, or peptide consists of 7 to about 50 amino acids comprising at least one peptide that is at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 100%, homologous with at least one Replikin peptide sequence identified in a drug-resistant pathogen or a drug-resistant bacterial pathogen. In one non-limiting embodiment, said at least one Replikin peptide sequence is at least one peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119. In a non-limiting embodiment, a homologue and a functional fragment of at least one Replikin peptide sequence includes SEQ ID NO(s): 120-122.

[00033] In a further non-limiting embodiment of the second aspect of the present invention, the isolated or synthesized protein, protein fragment, polypeptide, or peptide

consists essentially of a Replikin peptide identified in a drug-resistant pathogen or drug-resistant bacterial pathogen. In a further non-limiting embodiment, the Replikin peptide sequence is at least one peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119. A further non-limiting embodiment provides a peptide consisting of any one of SEQ ID NO(s): 1-21, 22-56, or 57-119.

[00034] Another non-limiting embodiment of the second aspect of the invention provides a biosynthetic composition comprising the protein, protein fragment, polypeptide, or peptide of an aspect of the invention. In a further non-limiting embodiment, the biosynthetic composition consists essentially of a Replikin peptide of a drug-resistant pathogen or drug-resistant bacterial pathogen or consists of a Replikin peptide of a drug-resistant pathogen or drug-resistant bacterial pathogen. In a non-limiting embodiment, an isolated protein, protein fragment, polypeptide, or peptide is chemically synthesized by solid phase methods.

[00035] A third non-limiting aspect of the present invention provides an immunogenic and/or blocking composition comprising at least one protein, protein fragment, polypeptide, or peptide of any one of the above-listed proteins, protein fragments, polypeptides, or peptides including and not limited to comprising at least one Replikin peptide sequence identified in a drug-resistant pathogen or drug-resistant bacterial pathogen or at least one homologue of said at least one Replikin peptide identified in a drug-resistant pathogen or drug-resistant bacterial pathogen or at least one functional fragment of at least one Replikin peptide sequence identified in a drug-resistant pathogen or drug-resistant bacterial pathogen. In a non-limiting embodiment of the third aspect of the present invention, the immunogenic and/or blocking compound comprises at least one peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119. In a further non-limiting embodiment, the immunogenic and/or blocking composition comprises at least one peptide consisting essentially of any one of SEQ ID NO(s): 1-21, 22-56, or 57-119. In further non-limiting embodiment, the immunogenic and/or blocking composition comprises at least one peptide consisting of any one of SEQ ID NO(s): 1-21, 22-56, or 57-119 or at least one functional fragment of any one of SEQ ID NO(s): 1-21, 22-56, or 57-119.

[00036] In a non-limiting embodiment, the at least one peptide consists of 50, 60, 70, 80, 90, 100, 150, or up to 200 amino acid residues. The at least one peptide comprises at least one Replikin peptide sequence, at least one homologue of at least one Replikin sequence, or at least one functional fragment of at least one Replikin sequence.

[00037] A fourth non-limiting aspect of the present invention provides a vaccine comprising at least one protein, protein fragment, polypeptide, or peptide of any one of the above-listed proteins, protein fragments, polypeptides, or peptides. In a non-limiting embodiment of the fourth aspect of the present invention, the vaccine comprises at least one peptide sequence of any one of SEQ ID NO(s): 1-21, 22-56, or 57-119, comprises at least one peptide sequence consisting essentially of any one of SEQ ID NO(s): 1-21, 22-56, or 57-119, and/or comprises at least one peptide sequence consisting of any one of SEQ ID NO(s): 1-21, 22-56, or 57-119, at least one homologue of any one of SEQ ID NO(s): 1-21, 22-56, or 57-119, or at least one functional fragment of any one of SEQ ID NO(s): 1-21, 22-56, or 57-119 or at least one Replikin peptide sequence identified in a drug-resistant pathogen or drug-resistant bacterial pathogen or at least one homologue or functional fragment of a Replikin peptide sequence identified in a drug-resistant pathogen or drug-resistant bacterial pathogen. In a non-limiting embodiment, the homologue or functional fragment is at least one of SEQ ID NO(s): 120-122.

[00038] In a further non-limiting embodiment of the fourth aspect of the present invention, the vaccine comprises a mixture of a plurality of peptide sequences of any of SEQ ID NO(s): 1-21, a mixture of a plurality of sequence of any of SEQ ID NO(s): 22-56, or a mixture of plurality of peptide sequences of any of SEQ ID NO(s): 57-119, and/or a mixture of a plurality of homologues of peptide sequences of any of SEQ ID NO(s): 1-21, 22-56, or 57-119. Such mixture may include homologues or functional fragments such as SEQ ID NO(s): 120-122. In a further non-limiting embodiment, the vaccine comprises a mixture of a plurality of peptide sequences of any of SEQ ID NO(s): 1-119. In a further non-limiting embodiment, the vaccine comprises a mixture of a plurality of peptide sequences consisting essentially of any one or more of SEQ ID NO(s): 1-21, 22-56, or 57-119. In a further non-limiting embodiment, the vaccine comprises a mixture of a plurality of peptide sequences consisting of any one or more of SEQ ID NO(s): 1-21, 22-56, or 57-119. In a further non-limiting embodiment, the vaccine comprises a mixture of a plurality of peptide sequences consisting of each of SEQ ID NO(s): 1-21, or each of SEQ ID NO(s): 22-56, or each of SEQ ID NO(s): 57-119, or each of SEQ ID NO(s): 1-119.

[00039] In another non-limiting embodiment of the fourth aspect of the invention, the vaccine comprises a mixture of Replikin peptides. In a non-limiting embodiment, the vaccine comprises an approximately equal molar mixture of the isolated or synthesized peptides of SEQ ID NO(s): 1-21, 22-56, or 57-119. In a further non-limiting embodiment, the vaccine

comprises approximately equal weight of the isolated or synthesized peptides of SEQ ID NO(s): 1-21, 22-56, or 57-119.

[00040] In a further non-limiting embodiment, the vaccine comprises a pharmaceutically-acceptable carrier and/or adjuvant. In a further non-limiting embodiment, the vaccine is for the treatment or prevention of a drug-resistant pathogen. In a further non-limiting embodiment, the vaccine is directed against Clostridium spp., Streptococcus spp., or Klebsiella spp. In a further non-limiting embodiment, the vaccine is directed again *Clostridium difficile, Streptococcus pneumoniae, or Klebsiella pneumoniae*. In a further non-limiting embodiment, the vaccine is directed against a bacterial pathogen such as a Gonococcus pathogen, a Tuberculosis pathogen, a Staphylococcus pathogen, or an E. coli pathogen. In a non-limiting embodiment, the pathogen is *Neisseria gonorrhoeae* or *Mycobacterium tuberculosis*. In a non-limiting embodiment, the pathogen is a fungus. In another non-limiting embodiment, the pathogen is *Candida albicans, Candida glabrata,* or Aspergillus. In another non-limiting embodiment, the pathogen is malaria or other parasitic pathogen.

[00041] A fifth non-limiting aspect of the invention provides an antibody, antibody fragment, or binding agent that preferentially binds to at least a portion of an amino acid sequence of at least one protein, protein fragment, polypeptide, or peptide comprising a peptide sequence that is at least 30%, 40%, 50%, 53%, 57%, 60%, 64%, 67%, 70%, 76%, 80%, 82%, 87%, 90%, 91%, 93%, or 95% or more homologous with at least one Replikin peptide sequence identified in a drug-resistant pathogen or a drug-resistant bacterial pathogen. In a further embodiment, the at least one Replikin peptide sequence is at least one peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119. In a further non-limiting embodiment, the antibody, antibody fragment, or binding agent preferentially binds at least one Replikin peptide sequence identified in a drug-resistant pathogen or a drug-resistant bacterial pathogen. In a further non-limiting embodiment, the antibody, antibody fragment, or binding agent preferentially binds within at least one Replikin peptide sequence. In a further non-limiting embodiment, the antibody, antibody fragment, or binding agent preferentially binds solely within at least one Replikin peptide sequence, homologue, or functional fragment thereof. In a further non-limiting embodiment, the antibody, antibody fragment, or binding agent preferentially binds an isolated or synthesized Replikin peptide. In a further non-limiting embodiment, the antibody, antibody fragment, or binding agent preferentially binds a homologue or functional fragment or a Replikin peptide sequence.

[00042] A sixth non-limiting aspect of the present invention provides a method of making a vaccine comprising: selecting at least one isolated or synthesized protein, protein fragment, polypeptide, or peptide comprising at least one peptide sequence that is at least 30%, 40%, 50%, 53%, 57%, 60%, 64%, 67%, 70%, 76%, 80%, 82%, 87%, 90%, 91%, 93%, 95%, or 100% homologous with at least one Replikin peptide sequence identified in drug-resistant pathogen or drug-resistant bacterial pathogen as a component of a vaccine; and making said vaccine. In a non-limiting embodiment, the method of making a vaccine comprises selecting at least one isolated or synthesized peptide of SEQ ID NO(s): 1-21, 22-56, or 57-119, as at least one component and making said vaccine with the at least one component. The vaccine may comprise at least one of SEQ ID NO(s): 120-122.

[00043] In another non-limiting embodiment, the method of making a vaccine comprises selecting at least two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or twenty-one or more isolated or synthesized Replikin peptide sequences identified in a drug-resistant pathogen or drug-resistant bacterial pathogen (or homologues thereof) and/or isolated or synthesized functional fragments of Replikin peptide sequences identified in a drug-resistant pathogen or drug-resistant bacterial pathogen. In a further embodiment, the isolated or synthesized Replikin peptide sequences or functional fragments of Replikin peptide sequences comprise at least one peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119, at least one functional fragment of at least one peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119, or at least one functional fragment of at least one Replikin peptide sequence identified in a drug-resistant pathogen or drug-resistant bacterial pathogen. In another non-limiting embodiment, the at least one isolated or synthesized protein, protein fragment, polypeptide, or peptide has the same amino acid sequence as at least one protein, protein fragment, polypeptide or peptide identified in a drug-resistant pathogen or drug-resistant bacterial pathogen up to seven days, one month, six months, one year, two years, or three years prior to making said vaccine.

[00044] A seventh non-limiting aspect of the present invention provides a method for preventing or treating drug-resistant pathogenic infection comprising administering at least one isolated or synthesized protein, protein fragment, polypeptide, or peptide comprising at least one peptide sequence to a subject, where the peptide sequence is at least 30%, 40%, 50%, 53%, 57%, 60%, 64%, 67%, 70%, 76%, 80%, 82%, 87%, 90%, 91%, 93%, 95%, or 100% homologous with at least one Replikin peptide identified in a drug-resistant pathogen

or a drug-resistant bacterial pathogen. In a further non-limiting embodiment, the Replikin peptide sequence is at least one peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119 or at least one homologue or functional fragment thereof. In a non-limiting embodiment, the at least one isolated or synthesized protein fragment, polypeptide, or peptide consists of at least one peptide sequence that is at least 30%, 40%, 50%, 53%, 57%, 60%, 64%, 67%, 70%, 76%, 80%, 82%, 87%, 90%, 91%, 93%, 95%, or more homologous with at least one of the peptide sequences of SEQ ID NO(s): 1-21, 22-56, or 57-119. In another non-limiting embodiment, the at least one isolated or synthesized peptide of SEQ ID NO(s): 1-21, 22-56, or 57-119 is administered to a human or other animal. In a further non-limiting embodiment the at least one Replikin peptide sequence is at least one peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119.

[00045] A non-limiting embodiment provides use of at least one peptide sequence that is at least 30%, 40%, 50%, 53%, 57%, 60%, 64%, 67%, 70%, 76%, 80%, 82%, 87%, 90%, 91%, 93%, 95%, or 100% homologous with at least one Replikin peptide identified in a drug-resistant pathogen or a drug-resistant bacterial pathogen in the manufacture of a medicament for preventing or treating drug-resistant pathogenic infection.

[00046] An eighth non-limiting aspect of the present invention provides nucleic acid sequences and compositions comprising nucleic acid sequences that encode for a Replikin peptide sequence isolated from or identified in a drug-resistant pathogen. In a non-limiting embodiment, the nucleic acid sequence encodes for at least one of SEQ ID NO(s): 1-119. In another non-limiting embodiment, the nucleic acid sequence is antisense to a fragment or to all of a sequence that encodes for a Replikin peptide sequence or is antisense to a sequence that encodes for any one or more of SEQ ID NO(s): 1-119 or a fragment thereof. A nucleic acid sequence may encode more than a Replikin peptide sequence or fragment thereof. Nucleic acid sequences may be comprised in a blocking agent or vaccine.

BRIEF DESCRIPTION OF THE DRAWINGS

[00047] Figure 1 illustrates percentage of specimens of Gonococcus (*Neisseria gonorrhoeae*) available at the PubMed website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) identified as having a Replikin concentration of greater than 4.0 per 100 amino acid residues for specimens identified as drug sensitive, specimens identified as resistant to the Cefixime antibiotic, specimens identified as resistant to Rifampicin antibiotic, specimens identified as resistant to Quinoline antibiotic, and

specimens identified as resistant to Tetracycline antibiotic. The percentage of specimens with Replikin concentrations of greater than 4.0 and the percentage of bacteria resistant to a particular antibiotic are observed to be proportional to the time in use of the particular antibiotic.

[00048] Figure 2 illustrates percentage of genomic sequences of *Mycobacterium tuberculosis* of five specific strains available at the PubMed website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) identified as having a Replikin concentration of greater than 4.0 per 100 amino acid residues. Percentage of sequences having a concentration of greater than 4.0 are individually illustrated for each of strains KZN 4207, KZN 1435, KZN R506, H37Ra, H37Rv, and KZN 605. Strain KZN 4207 is identified as fully drug sensitive, strain KZN 1435 is identified as drug resistant, strain KZN R506 is identified as drug resistant, and strain KZN 605 is identified as extremely drug resistant. Strains H37Ra and H37Rv are identified as attenuated virulence and virulent, respectively. Number of sequences analyzed for each of strains KZN 4207, KZN 1435, KZN R506, H37Ra, H37Rv, and KZN 605 were 3,883, 2,610, 1,278, 3,350, 1,217, and 2,592, respectively.

[00049] Figure 3 illustrates percentage of specimens of carbapenem-resistant *Klebsiella pneumoniae* available at the PubMed website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) identified as having a Replikin concentration of greater than 4.0 per 100 amino acid residues for specimens isolated in years 2007 through 2012. An outbreak of carbapenem-resistant *Klebsiella pneumoniae* struck the U.S. National Institutes of Health Clinical Center in 2011 affecting 18 patients. Eleven of the 18 patients died of the infection.

[00050] Figure 4 illustrates percentage of specimens of drug-resistant *Clostridium difficile* available at the PubMed website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) identified as having a Replikin concentration of greater than 4.0 per 100 amino acid residues for specimens isolated in years 1998 through 2011.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

[00051] As used herein, a "Replikin sequence" is an amino acid sequence of 7 to about 50 amino acids comprising (1) a first lysine residue located six to ten residues from a second

lysine residue, (2) at least one histidine residue; and (3) at least 6% lysine residues. A Replikin sequence may have a lysine residue on one end of the sequence and a lysine residue or histidine residue on the other end of the sequence. For the purpose of determining Replikin concentration, a Replikin sequence is the shortest amino acid sequence of 7 to 50 amino acid residues comprising (1) a first lysine residue located six to ten residues from a second lysine residue, (2) at least one histidine residue; and (3) at least 6% lysine residues. For purposes of determining Replikin concentration, the Replikin sequence may comprise any number of lysine residues and any number of histidine residues so long as any two lysine residues and any one histidine residue reflect the requirements of the Replikin sequence. As a result, a Replikin sequence counted as part of the Replikin concentration of a sequence of amino acid residues may comprise overlapping Replikin sequences.

[00052] The term "Replikin sequence" can also refer to a nucleic acid sequence encoding an amino acid sequence having 7 to about 50 amino acids comprising:

(1) at least one lysine residue located six to ten amino acid residues from a second lysine residue;

(2) at least one histidine residue; and

(3) at least 6% lysine residues,

wherein the amino acid sequence may have a lysine residue on one end of the sequence and a lysine residue or histidine residue on the other end of the sequence or may be the shortest amino acid sequence having any two lysine residues and any one histidine residue reflecting the requirements of the Replikin sequence.

[00053] As used herein, "Replikin Count™" or "Replikin Concentration" refers to the number of Replikin sequences per 100 amino acids in a protein, protein fragment, virus, or organism. A higher Replikin concentration in a first strain of a virus or organism has been found to correlate with more rapid replication of the first virus or organism as compared to a second, earlier-arising or later-arising strain of the virus or organism having a lower Replikin concentration. Replikin concentration is determined by counting the number of Replikin sequences in a given amino acid sequence or in a nucleic acid that encodes at least one Replikin peptide sequence. One aspect of the current invention provides a method of diagnosing the drug sensitivity or drug resistance of a pathogen comprising determining if a Replikin concentration is greater than 4.0 per 100 amino acid residues. A Replikin concentration of greater than 4.0 per 100 amino acid residues denotes drug resistance and a concentration or 4.0 or less per 100 amino acid residues denotes drug sensitivity.

[00054] As used herein, "drug-resistant" pathogens reflect populations of a pathogen that does not respond to treatment with an antimicrobial therapy at the same therapeutic level as other populations of the same pathogen at previous times or in different geographical areas.

[00055] As used herein, the term "peptide" or "protein" refers to a compound of two or more amino acids in which the carboxyl group of one amino acid is attached to an amino group of another amino acid via a peptide bond.

[00056] As used herein, "isolated" or "synthesized" peptide or biologically-active portion thereof refers to a peptide that is, after purification, substantially free of cellular material or other contaminating proteins or peptides from the cell or tissue source from which the peptide is derived, or substantially free from chemical precursors or other chemicals when chemically synthesized by any method, or substantially free from contaminating peptides when synthesized by recombinant gene techniques. A protein or peptide may be isolated *in silico* from nucleic acid or amino acid sequences that are available through public or private databases or sequence collections.

[00057] An "encoded" or "expressed" protein, protein sequence, protein fragment sequence, or peptide sequence is a sequence encoded by a nucleic acid sequence that encodes the amino acids of the protein or peptide sequence with any codon known to one of skill in the art now or hereafter. It should be noted that it is well-known in the art that, due to redundancy in the genetic code, individual nucleotides can be readily exchanged in a codon and still result in an identical amino acid sequence. As will be understood by one of skill in the art, a method of identifying a Replikin amino acid sequence also encompasses a method of identifying a nucleic acid sequence that encodes a Replikin amino acid sequence wherein the Replikin amino acid sequence is encoded by the identified nucleic acid sequence. A nucleic acid sequence that encodes a Replikin peptide sequence or a homologue or fragment thereof encodes only the Replikin peptide sequence or a homologue or fragment thereof and not more than the Replikin peptide sequence or a homologue or fragment thereof.

[00058] As used herein, "conserved" or "conservation" refers to conservation of particular amino acid residues due to lack of substitution.

[00059] As used herein, "outbreak" is an increase in virulence, morbidity, and/or mortality in a pathogenic disease or an expansion in the population of pathogen as compared to a baseline of an earlier occurring epidemiological pattern of infection in the same disease. One of ordinary skill in the art will know how to determine an epidemiological baseline.

[00060] As used herein, an "isolate" is any organism isolated from a natural source wherein a natural source includes, but is not limited to, a reservoir of an organism or virus, a vector of an organism or virus, or a host of an organism or virus. "Obtaining," "isolating," or "identifying" an isolate is any action by which an amino acid or nucleic acid sequence within an isolate is obtained including, but not limited to, isolating an isolate and sequencing any portion of the genome or protein sequences of the isolate, obtaining any nucleic acid sequence or amino acid sequence of an isolate, wherein the nucleic acid sequence or amino acid sequence may be analyzed for Replikin concentration, or any other means of obtaining the Replikin concentration of a virus isolated from a natural source at a time point or within a time period. Isolated or related words may also mean: identified within such a Replikin sequence identified within a larger polypeptide.

[00061] "Functional derivatives" of the Replikin sequences described herein are fragments, variants, analogs, or chemical derivatives of Replikin sequences that retain at least a portion of the immunological cross reactivity with an antibody specific for the Replikin sequence. A fragment of the Replikin peptide refers to any subset of the molecule. A "functional fragment" of the Replikin peptide or a Replikin Peak Gene is any subset of the molecule that retains at least a portion of immunological cross reactivity with an antibody specific for the Replikin peptide or Replikin Peak Gene. Variant peptides may be made by direct chemical synthesis, for example, using methods well known in the art. An analog of a Replikin peptide sequence to a non-natural protein substantially similar to either the entire protein or a fragment thereof. Chemical derivatives of a Replikin peptide sequence contain additional chemical moieties not normally a part of the peptide or peptide fragment.

[00062] As used herein, "homologue" or "homologous" or "homology" or "sequence identity" are used to indicate that an amino acid sequence or nucleic acid sequence exhibits substantial structural or functional equivalence with another sequence. Any structural or functional differences between sequences having sequence identity or homology will be *de minimus;* that is, they will not affect the ability of the sequence to function as indicated in the desired application. Structural differences are considered *de minimus* if there is a significant amount of sequence overlap or similarity between two or more different sequences or if the different sequences exhibit similar physical characteristics even if the sequences differ in length or structure. Such characteristics include, for example, the ability to hybridize under defined conditions, or in the case of proteins and peptides, immunological cross-reactivity,

similar enzymatic activity, *etc*. The ordinary skilled practitioner can readily determine each of these characteristics by art-known methods.

[00063] To determine the percent identity or percent homology of two sequences, the sequences are aligned for optimal comparison purposes (*e.g.,* gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). In a non-limiting embodiment, at least 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97%, 99% or more of the length of a reference sequence is aligned for comparison purposes. The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position (as used herein amino acid or nucleic acid "identity" is equivalent to amino acid or nucleic acid "homology"). The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences as compared to the total length of the sequence identified as a reference sequence.

[00064] As used herein, a "Replikin Peak Gene" (RPG) means a segment of a genome, protein, segment of protein, or protein fragment in which an expressed gene or gene segment has a highest concentration of continuous, non-interrupted and overlapping Replikin sequences (number of Replikin sequences per 100 amino acids) when compared to other segments or named genes of the genome. Generally, a whole protein or gene or gene segment that contains the amino acid portion having the highest concentration of continuous Replikin sequences is also referred to as the Replikin Peak Gene. More than one RPG may be identified within a gene, gene segment, protein, or protein fragment. An RPG may have a terminal lysine or a terminal histidine, two terminal lysines, or a terminal lysine and a terminal histidine. For diagnostic, therapeutic and preventive purposes, an RPG may have a terminal lysine or a terminal histidine, two terminal lysines, or a terminal lysine and a terminal histidine or may likewise have neither a terminal lysine nor a terminal histidine so long as the terminal portion of the RPG contains a Replikin sequence or Replikin sequences defined by the definition of a Replikin sequence, namely, an amino acid sequence having 7 to about 50 amino acids comprising:

(1) at least one lysine residue located six to ten amino acid residues from a second lysine residue;

(2) at least one histidine residue; and

(3) at least 6% lysine residues.

Further, for diagnostic, therapeutic, preventive and predictive purposes, an RPG may include the protein or protein fragment that contains an identified RPG. For predictive purposes, a Replikin concentration in the RPG may be used to track changes in virulence and lethality. Likewise the RPG may be used as an immunogenic compound or as a vaccine. Whole proteins or protein fragments containing RPGs are likewise useful for diagnostic, therapeutic and preventive purposes, such as, for example, to be included in immunogenic compounds, vaccines and for production of therapeutic or diagnostic antibodies.

[00065] As used herein, the term "continuous Replikin sequences" means a series of two or more Replikin sequences that are overlapped and/or are directly covalently linked.

[00066] As used herein, an increase or decrease in "virulence" includes an increase or decrease in virulence, morbidity, lethality, host mortality, rate of replication, rate of distribution, and/or expansion of a drug-resistant pathogen.

[00067] As used herein, "geographic region" or similar term is an area differentiated from another area by space. For example, China is a geographic region that may be differentiated from the geographic region of India. Likewise a geographic region may be a hospital, a clinic, a town, or city, or continent or any area differentiable from another area. A geographic region may encompass the entire earth if an isolate or plurality of isolates from a given time period is compared to isolates from another time period over the entire earth and no geographic differentiation is undertaken for the comparison.

[00068] As used herein, the term "preferentially binds" or "specifically binds" and related terms referencing the interaction of a binding molecule such as, for example, an antibody, and the structure to which it binds (antigen) means that the binding molecule preferentially recognizes the structure to which it binds even when present among other molecules (such as in a mixture of molecules). Specific or preferential binding of a binding molecule to a binding structure or an immunogenic portion of a binding structure is specific and preferential when the binding molecule binds to the structure or portion thereof and does not bind with the same level of affinity to other structures. Binding affinity may be determined by one of ordinary skill in the art using, for example, BIACORE, enzyme-linked immunosorbent assays, or radioimmuno assays. A binding molecule may cross-react with

related antigens and preferably does not cross-react with affinity to unrelated antigens. Binding between a binding molecule and the structure to which it binds may be mediated by covalent or non-covalent attachment, or both.

Replikin concentrations of greater than 4.0 correlate with drug-resistance in pathogens

[00069] Applicants analyzed Replikin concentrations in 130,725 genomic sequences from several pathogenic bacteria published at the PubMed website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). In a surprise discovery providing for diagnosis of development of drug resistance in a pathogen and for design and manufacture of therapies against drug-resistant pathogens, the applicants determined that when drug-resistance develops in a population of bacteria, there is a marked increase in genomic Replikin concentrations (number of genomic Replikin sequences per 100 amino acids at $p<0.001$). Replikin concentrations of 0.1 to 4.0 were found in antibiotic-sensitive bacteria while antibiotic-resistant bacteria isolates were found to have Replikin concentrations of from 4.1 to 50.

[00070] Prior to the present invention, Replikin sequences had not been associated with drug resistance in pathogens. Further, no structures of any kind in infectious organisms had been described that correlate quantitatively and temporally with increases in drug resistance in pathogens or with outbreaks of drug-resistant pathogens. In fact, the global health community (including the chief medical officer of the U.K.) has acknowledged a lack of drug candidates against resistant pathogens and a lack of targets for such drug candidates. *See, e.g.,* Nature News Blog (11 Mar 2013) (http://blogs.nature.com/news/2013/03/drug-resistant-bacteria-and-lack-of-new-antibiotics-pose-catastrophic-threat.html).

[00071] The applicants' identification of an increase in Replikin concentration in drug-resistant pathogens provides a diagnostic marker for the development of resistance and a target for therapies against such resistance. As resistance increases in the population of a specific pathogen, including a bacterium, fungus, or parasite, the percent of all isolated specimens having an increase in Replikin concentration in genomic sequences increases. The percent of the specific pathogenic population at a given time that has a Replikin concentration of greater than 4.0 increases from zero percent to as much as 95% of the circulating population. *See, e.g.* Figure 1. It was also found that in the evolution of each bacterial genome, as new Replikin sequences are added to or disappear from the genome, certain individual Replikin sequences remain conserved for decades. This discovery provides for long-acting vaccines. This genomic-structural property shared by different antibiotic-

resistant bacterial strains presents a completely new platform in bacterial genomic structure for specific early diagnosis, vaccines, and therapies.

[00072] Before this work on bacteria, earlier studies on viruses, including influenza, found that when virus genomes contained increasing concentrations of Replikin sequences, outbreaks of these specific viruses, and the geographic sites of these outbreaks were predicted one to two years in advance of the outbreak. More than seven out of seven such correct predictions have been made and published in prospective trials in the past five years, including a 2008 prediction of the coming 2009 H1N1 influenza pandemic. *See, e.g.* "Prediction of specific virus outbreaks made from the increased concentration of a new class of virus genomic peptides, Replikins," Nature Precedings (22 Aug 2011). An additional (eighth) prediction was noted in 2012 and four more such correct predictions have followed. Retrospectively, an association was found in all influenza pandemics and major outbreaks in the past century. *See, e.g.,* WO 2009/132209, Figures 7 and 8. A resulting H5N1 vaccine, produced in seven days and shipped freeze-dried, has been successful in poultry in blocking virus entry, replication and excretion. *See, e.g.,* WO 2010/123591, Figure 1. A resulting vaccine in Taura Syndrome Virus has also demonstrated protection of 91% of shrimp tested against the fatal taura syndrome virus. *See, e.g.,* WO 2008/156914, Figures 1 and 2.

Vaccine against drug-resistant pathogens

[00073] One aspect of the present invention provides therapies, including vaccines, against drug-resistant pathogens. One non-limiting embodiment of the one aspect is a vaccine against a drug-resistant bacteria, fungus, or parasite. Such drug-resistant pathogens include, and are not limited to, Tuberculosis, Gonococcus, Klebsiella, Clostridium, Streptococcus, Staphylococcus, E. coli, Candida, Aspergillus, and malaria.

[00074] A vaccine may comprise at least one protein, protein fragment, polypeptide, or peptide comprising at least one Replikin sequence identified in a drug-resistant pathogen or at least one sequence that is at least 30%, 40%, 50%, 53%, 57%, 60%, 64%, 67%, 70%, 76%, 80%, 82%, 87%, 90%, 91%, 93%, 95% or more homologous with at least one Replikin sequence identified in a drug-resistant pathogen. The vaccine may further comprise a mixture of proteins, protein fragments, polypeptides, or peptides comprising at least one Replikin sequence identified in a drug-resistant pathogen or at least one homologue of the at least one Replikin sequence. The vaccine may comprise a peptide that consist essentially of a Replikin sequence identified in a drug-resistant pathogen or homologue or functional

fragment thereof or may comprise a peptide that consists of a Replikin sequence identified in a drug-resistant pathogen or homologue or functional fragment thereof.

[00075] A vaccine comprising any one or more peptide sequences of SEQ ID NO(s): 1-21 is provided herein against Clostridium species, including *Clostridium difficile*. SEQ ID NO: 120 is provided as an example of a homologue and functional fragment of SEQ ID NO(s): 16 and 17 and may be comprised in a vaccine against Clostridium species. A vaccine comprising any one or more peptide sequences of SEQ ID NO(s): 22-56 is provided herein against Streptococcus species including *Streptococcus pneumoniae*. SEQ ID NO: 121 is provided as an example of a homologue and functional fragment of SEQ ID NO(s): 40 and 41 and may be comprised in a vaccine against Streptococcus species. A vaccine comprising any one or more peptide sequences of SEQ ID NO(s): 57-119 is provided herein against Klebsiella species including *Klebsiella pneumoniae*. SEQ ID NO: 122 is provided as an example of a homologue and functional fragment of SEQ ID NO(s): 105 and 106 and may be comprised in a vaccine against Klebsiella species.

[00076] These vaccines are designed to generate an immune or blocking response in the subject that antagonizes infectivity, replication, and shedding of these drug-resistant bacteria. Any bacteria that are not blocked at entry into the subject will be blocked from replication within the subject or in association with tissue of the subject.

Vaccine Design and Manufacture

[00077] Observations of specific Replikin sequences and their change in concentration in genomic and/or proteomic sequences of specimens of drug-resistant pathogens provides specific quantitative early chemical correlates of increased drug-resistance and outbreaks of drug-resistant populations and provides for production and timely administration of vaccines tailored specifically to treat the prevalent emerging or re-emerging drug-resistant strain in a particular region of the world, a country, a province, a town, school, hospital, other institution, or any geographical location or region. Synthesis of these vaccines may be accomplished in seven days or less, which allows for administration of vaccines that are a best fit for a particular virulent strain of bacteria.

[00078] By analyzing the protein sequences of isolates of a drug-resistant bacterial, fungal, or parasitic pathogen for the presence, concentration and/or conservation of Replikin sequences and by comparing changes in Replikin concentrations among a population or sub-population, outbreaks and other changes in virulence and mortality, as well as increases in drug resistance, is diagnosed and treatments are developed. Furthermore, the severity of such

outbreaks can be significantly lessened by administering a peptide or polypeptide vaccine based on the Replikin sequences found to be most abundant or shown to be on the rise in drug-resistant isolates over a given time period, such as about one to about three years. A non-limiting embodiment of the present invention provides a method a making a vaccine comprising identifying a population having an increasing number of specimens with Replikin concentrations above 4.0, identifying at least one Replikin sequence in at least one specimen with a Replikin concentration above 4.0, and manufacturing a vaccine comprising the at least one Replikin sequence or a homologue or functional fragment thereof.

[00079] A peptide vaccine of the invention may include a single Replikin peptide sequence identified in a pathogen or pathogen population (or homologue or functional fragment thereof) or may include a plurality of Replikin sequences observed in particular strains (or homologues or functional fragments thereof). A peptide vaccine may comprise a Replikin peptide sequence or plurality of Replikin peptide sequences, it may consist essentially of a Replikin peptide sequence or a plurality of Replikin peptide sequences, or may consist of a Replikin peptide sequence or plurality of Replikin peptide sequences. A vaccine may include one or more conserved Replikin peptide sequences in combination with one or more new Replikin peptide sequences or may be based on new Replikin peptide sequences in a population increasing in drug-resistance. A vaccine may comprise a protein or protein fragment comprising a Replikin peptide sequence or may comprise a peptide comprising a Replikin peptide sequence or may comprise a peptide consisting essentially of a Replikin peptide sequence or may be a Replikin sequence. A protein or protein fragment for inclusion in a vaccine may be identified by identifying a Replikin peptide sequence within a protein or protein fragment or a homologue of a Replikin peptide sequence within a protein or protein fragment. A vaccine may comprise a pharmaceutically acceptable carrier and/or adjuvant and/or excipient.

[00080] Polypeptides, protein fragments, or peptides comprising a Replikin peptide sequence, consisting essentially of a Replikin peptide sequence, or consisting of a Replikin peptide sequence can be synthesized by any method, including chemical synthesis, biosynthetic synthesis, solid-phase synthesis, or recombinant gene technology, and may include non-Replikin sequences. Vaccine composition may comprise a pharmaceutically acceptable carrier and/or adjuvant and/or excipient. Among the Replikin peptide sequences for use as immunogenic or blocking targets in a bacterial or pathogenic vaccine are those

Replikin sequences observed to "re- emerge" after an absence from the amino acid sequence for one or more years.

[00081] The vaccines of one aspect of the present invention can be administered alone or in combination with antimicrobial drugs, such as gancyclovir; interferon; interleukin; M2 inhibitors, such as, amantadine, rimantadine; neuraminidase inhibitors, such as zanamivir and oseltamivir; and the like, as well as with combinations of antimicrobial drugs.

[00082] One vaccine of one aspect of the present invention may be administered to any animal capable of producing antibodies in an immune response or capable of mounting an antimicrobial response such as an innate immune response or a mucosal immune response. For example, a vaccine may be administered to a mouse, a rabbit, a chicken, a shrimp, a pig, or a human. Because of the universal nature of Replikin sequences, a vaccine of the invention may be directed at a range of strains of a drug-resistant pathogen or a particular strain of pathogen. The pathogen may be a bacterium, a fungus, a parasite, or any other infectious pathogen.

[00083] The Replikin peptide sequences reflecting one aspect of the invention, alone or in various combinations reflect immunogenic or blocking targets that may be administered to a subject, in a non-limited embodiment, by *i.v.* intramuscular injection, by mouth, or by spray inhalation, intranasal administration, or intraocular administration (or any other route known to one of skill in the art). The peptides or polypeptides comprising the peptide sequences are administered in order to stimulate the immune system of the subject to produce antibodies to the peptide and/or in order to produce a blocking action. Generally the dosage of peptides or protein fragments is in the range of from about 0.01 μg to about 500 mg, about 0.05 μg to about 200 mg, or about 0.075 μg to about 30 mg, from about 0.09 μg to about 20 mg, from about 0.1 μg to about 10 mg, from 10 μg to about 1 mg, and from about 50 μg to about 500 μg. The skilled practitioner can readily determine the dosage and number of dosages needed to produce an effective immune response.

[00084] Another aspect of the invention provides nucleic acid sequences and compositions comprising nucleic acid sequences that encode for the above-discussed proteins, protein fragment, polypeptides, and peptides including, for example, nucleic acid sequences that encode for a Replikin peptide sequence identified in a drug-resistant pathogen. In a further embodiment, the nucleic acid sequence may encode for any one or more of SEQ ID NO(s): 1-119 or a fragment thereof. These sequences may be comprised in a blocking agent or vaccine.

[00085] In another aspect of the invention, isolated or synthesized proteins, protein fragment, polypeptides, or peptides comprising Replikin peptide sequences may be used to generate antibodies, which may be used, for example to provide passive immunity in an individual or for diagnostic purposes. *See, e.g.* WO 2006/088962 and WO 2008/143717 (each incorporated herein by reference in their entirety).

Replikin sequence homologues as targets for controlling drug-resistant replication

[00086] Homologues of Replikin sequences may be comprised in vaccine against replication of drug-resistant pathogens. In a non-limiting embodiment, a vaccine may comprise a peptide that is 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 97%, 99% or more homologous with a Replikin sequence identified in a drug-resistant pathogen.
For example, in *C. difficile*, KGGGQWEHK (SEQ ID NO: 18) is 67% homologous with HYHRSNKGGGQWEHK (SEQ ID NO: 16) since SEQ ID NO: 18 shares 10 exact residues with the C-terminal end of SEQ ID NO: 16 and SEQ ID NO: 16 is fifteen residues long. As a result, SEQ ID NO: 18 is available as a peptide in a vaccine as a homologue of SEQ ID NO: 16. Likewise, HRSNKGGGQWEHKK (SEQ ID NO: 17) is 87% homologous with SEQ ID NO: 16 since SEQ ID NO: 17 shares thirteen exact residues with SEQ ID NO: 16, which is 15 residues long. Also, KTHRGAAK (SEQ ID NO: 2) is 80% homologous with SEQ ID NO: 1.

[00087] Other examples of homology (among many in this specification) include SNKGGGQW (SEQ ID NO: 120), which is 53% homologous with SEQ ID NO: 16 and 57% homologous with SEQ ID NO: 17. Any functional homologue of a Replikin sequence may be used as a target for controlling replication in a drug-resistant pathogen. A functional homologue allows the immune system to target the structure and function of a Replikin sequence in the drug-resistant pathogen.

[00088] In streptococcus, for example, HLGLTKADMLYPRK (SEQ ID NO: 41) is 82% homologous with HNGHLGLTKADMLYPRK (SEQ ID NO: 40). Likewise, HLGLTKADMLYPR (SEQ ID NO: 121) is 93% homologous with SEQ ID NO: 41 and 76% homologous with SEQ ID NO: 40. Any functional homologue of a Replikin sequence in streptococcus may be used as a target for controlling drug-resistant replication. A functional homologue allows the immune system to target the structure and function of a Replikin sequence in the drug-resistant pathogen.

[00089] In Klebsiella, for example, KGYDVKATHK (SEQ ID NO: 106) is 91% homologous with KMKGYDVKATH (SEQ ID NO: 105). GYDVKAT (SEQ ID NO: 122) is

70% homologous with SEQ ID NO: 106 and 64% homologous with SEQ ID NO: 105. Any functional homologue of a Replikin sequence in Klebsiella may be used as a target for controlling drug-resistant replication. A functional homologue allows the immune system to target the structure and function of a Replikin sequence in the drug-resistant pathogen.

Vaccine formulations

[00090] A vaccine may be formulated with a pharmaceutically acceptable excipient, carrier, or adjuvant. One pharmaceutically acceptable carrier or excipient is water. Excipients, carriers, or adjuvants may include, but are not limited to, excipients, carriers and adjuvants known to those of skill in the art now or hereafter.

[00091] The compositions of an aspect of the invention may be formulated for delivery by any available route including, but not limited to parenteral (e.g., intravenous), intradermal, subcutaneous, oral, nasal, bronchial, ophthalmic, transdermal (topical), transmucosal or any other routes. As used herein the language "pharmaceutically acceptable carrier" includes solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like, compatible with pharmaceutical administration. Supplementary active compounds can also be incorporated into the compositions.

[00092] A pharmaceutical composition is formulated to be compatible with its intended route of administration. Solutions or suspensions used for intranasal, intraocular, spray inhalation, parenteral (e.g., intravenous), intramuscular, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water (for dermal, nasal, or ocular application, spraying, or injection), saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; buffers such as acetates, citrates or phosphates and agents for the adjustment of tonicity such as sodium chloride or dextrose. pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. Preparations may be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00093] Pharmaceutical compositions suitable for injectable use typically include sterile aqueous solutions (water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition should be

sterile and should be fluid to the extent that easy syringability exists. Preferred pharmaceutical formulations are stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. In general, the relevant carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol, and the like), and suitable mixtures thereof. Synthetic peptides may be carried in sterile water with no additional materials.

[00094] Sterile injectable solutions can be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above.

[00095] Administration of the vaccine via any method may produce an immune or blocking response in the animal or human, it may further produce an antibody response in the animal or human. In a further non-limiting embodiment, the vaccine may produce a protective effect in the animal or human. For example, the vaccine of the present invention may be administered to a rabbit, a chicken, a shrimp, a pig, a ferret, a human, or any animal capable of an immune or blocking response. Because of the universal nature of Replikin sequences, a vaccine of the invention may be directed at a range of strains of drug-resistant pathogens, including drug-resistant bacterial pathogens.

Stimulating the immune system

[00096] One aspect of the present invention is a method of stimulating the immune system of a subject with at least one compound comprising at least one Replikin sequence identified in a drug-resistant bacterium or other pathogen or at least one isolated or synthesized homologue of at least one Replikin sequence identified in a drug-resistant bacterium or other pathogen. The at least one Replikin sequence of the compound reflects an immunogenic target against which the immune system of the subject responds. Because at least a functional portion of the immunogenic structure of the target is maintained in a functional fragment of the at least one Replikin sequence, a functional fragment of the Replikin sequence is likewise a target against which the immune system of the subject responds. The compound may comprise a protein comprising the at least one Replikin sequence or functional fragment thereof, a protein fragment, a polypeptide, or a peptide comprising the at least one Replikin sequence or homologue or functional fragment thereof. The compound

may comprise more than one protein, protein fragment, polypeptide or peptide. The compound may further be a composition of a plurality of synthesized or isolated Replikin sequences.

Immunogenic fragments of Replikin sequences identified in drug-resistant pathogens and their utilities

[00097] Another non-limiting aspect of the present invention provides an isolated or synthesized peptide that is an immunogenic fragment of a Replikin sequence of a drug-resistant pathogen. Such fragments are functional fragments. Immunogenic fragments of a Replikin sequence are fragments that provide at least a portion of cross-reactivity with an antibody or antibody fragment against the Replikin sequence. One embodiment of an aspect of the present invention provides an isolated or synthesized immunogenic (or functional) fragment of at least one Replikin sequence of a drug-resistant pathogen as a target for control of replication of the pathogen. One embodiment provides the isolated or synthesized immunogenic fragment as an antigen for stimulating the immune system of a subject, including but not limited to a human, to produce antibodies against the fragment. Another embodiment of an aspect of the invention provides the isolated or synthesized immunogenic fragment as a vaccine or as a component of a vaccine in combination with an acceptable pharmaceutical carrier or in combination with other proteins, peptides, immunogenic substances, and/or adjuvant(s).

Advance Replikin-Based Information on Drug-resistant Pathogenic Outbreaks Provides For Rapid Production of Vaccines

[00098] Advance information concerning Replikin peptides in expanding strains of drug-resistant pathogen allows for the rapid production of specific, effective, synthetic vaccines using one, or a combination, of Replikin peptide sequences or proteins, protein fragments, polypeptides, or peptides comprising Replikin peptide sequences. Within these peptide and polypeptide sequences the Replikin peptide sequence, a homologue of the Replikin peptide sequence, or a functional fragment of the Replikin peptide sequence or multiple Replikin peptide sequences provide for targets against replication of the pathogen and against the drug-resistant mechanisms of the pathogen.

[00099] Synthetic Replikin-targeting vaccines have been demonstrated in rabbits, chickens, and shrimp. *See, e.g.,* Example 1 herein, Examples 6 and 7 of U.S. Appln. Ser. No. 11/355,120, filed February 16, 2006, and Example 2 of U.S. Appln. Ser. No. 12/108,458, filed April 23, 2008. For example, a mixture of Replikin peptides administered orally to

shrimp provided up to a 91% protective effect for shrimp challenged with taura syndrome virus. Taura syndrome virus is an often-lethal rapidly replicating pathogen that has a significant negative impact on the shrimp industry.

[000100] Synthetic Replikin vaccines have also been demonstrated in the H5N1 strain of influenza virus in chickens. For example, in a test of chickens administered a mixture of twelve H5N1 Replikin peptides from the hemagglutinin and pB1 gene areas intranasally, intraocularly, and by spray inhalation and challenged with low pathogenic H5N1 influenza virus isolated from a black duck in the state of North Carolina in the United States, a protective effect was observed at both the entry site of influenza (diminished antibody production in the serum was observed as compared to a control) and at excretion sites of influenza (influenza virus was not observed excreted in feces or saliva from treated chickens as compared to a control).

[000101] Administration of Replikin peptides in both shrimp and chickens appears to have provided a notable measure of mucosal immunity. For example, in Example 2 of U.S. Appln. Ser. No. 12/108,458, a mixture of Replikin peptides was administered by mouth to shrimp later challenged with taura syndrome virus. The 91% protective effect of the vaccine is expected to have been a result, at least in part, of a mucosal immune-like response in the gut of the shrimp.

[000102] Likewise, in chickens, the administration of a mixture of Replikin peptides provided a protective effect against entry of the H5N1 virus. For example, as may be seen in Example 1 of U.S. Appln. Ser. No. 12/581,112, filed October 16, 2009 (incorporated herein by reference), three of six vaccinated chickens, when inoculated with H5N1 virus, produced no measurable amount of antibodies against H5N1 in their serum. Instead, the virus was apparently blocked by mucosal immunity from even entering the chickens' system. Some virus apparently entered the system of the chickens but was then blocked intracellularly. While the applicants do not wish to be bound by theory, the virus may have been blocked in its intracellular transport to the RNA or in synthesis of virus on RNA or in transport from the RNA to excretion. Wherever the block occurs, the fact is that the examination of the excreta of the chicken showed complete absence of virus. For those three chickens in which a serum immune response was measured (that is, virus did enter their system), the vaccine additionally provided a protective effect against replication of the virus in the chickens' system (no virus was excreted in the feces or saliva of the chickens). As such, mucosal

immunity, in addition to other immunities, is an important aspect of the immunity imparted by Replikin-based vaccines.

Conserved Replikin sequences

[000103] Replikin peptides in general are seen to be conserved across strains of drug-resistant bacteria. Example 7 herein provides an illustration of this conservation. The key amino acid residues that provide for the Replikin sequence structure are the lysine and histidine residues, wherein a Replikin sequence has at least one lysine on one terminus and at least one lysine or one histidine on the other terminus, at least one lysine that is six to ten residues from at least one other lysine, at least one histidine, and at least six percent lysine residues in total between the terminal lysine and the terminal lysine or histidine.

[000104] As may be seen in Figure 10 of WO 2005/104754, when conserved homologous Replikin sequences are aligned one on top of the other over time, it is most apparent that fixed and conserved portions of the structure of Replikin sequences align in a series of posts or girders that illustrate, like the structure of a building, how key conserved amino acids provide constancy for the survival of a virus over time as it mutates to avoid immune recognition in its prospective host but maintains key functional genetic structures that provide for continued replication of the virus. These key functional genetic structures provide targets antagonized by Replikin-based therapies including therapies against antimicrobial resistant pathogens.

[000105] The same mechanism of conservation is seen in drug-resistant bacteria as they accumulate mutations to overcome antibiotic challenges. As a result, one aspect of the invention provides for the conservation of the lysine residues and histidine residues of the Replikin sequences of a bacterial genome as the bacterial population develops antibiotic resistance. The lysine residue and histidine residue structures therefore provide targets for attacking the replicating and drug-resistance mechanisms of the pathogen. As a result, conservation of the lysine and histidine residues that define a Replikin peptide sequence provides for a target against drug-resistant pathogens. Sequences that reflect changes in all residues except the lysine and histidine residues that define a Replikin peptide sequence are therefore particularly useful as vaccine targets and reflect a non-limiting embodiment of an aspect of the invention. A homologue of SEQ ID NO: 1, therefore, is any sequence with a lysine at position one, a histidine at position five, and a lysine at position ten. Likewise, a homologue of SEQ ID NO: 22 is any sequence with a lysine at position one, a lysine at position eleven and a histidine at position fourteen. A homologue of SEQ ID NO: 57 is any

sequence with a lysine at position one, a lysine at position eight, and a histidine at position sixteen. These are provided as examples and one of ordinary skill can determine similar homologues of any identified Replikin sequence, including any of SEQ ID NO(s): 1-119. Any of these homologues provides excellent targets for controlling replication of drug-resistant pathogens.

[000106] Conserved Replikin sequences in isolates of carbapenem-resistant Klebsiella are provided in Example 7. All conserved Replikin sequences isolated from drug-resistant pathogens provide excellent targets for control of replication of drug-resistant pathogens such as a Gonococcus pathogen, a Tuberculosis pathogen, a Klebsiella pathogen, a Clostridium pathogen, a Streptococcus pathogen, a Staphylococcus pathogen, an E. coli pathogen, *Neisseria gonorrhoeae*, *Mycobacterium tuberculosis*, *Klebsiella pneumoniae, Clostridium difficile,* or *Streptococcus pneumonia* and including carbapenem-resistant Klebsiella.

Methods of determining/diagnosing drug-resistant pathogens using Replikin concentration analysis

[000107] One aspect of the present invention provides a method of diagnosing and/or determining the drug sensitivity or drug resistance of a pathogen. Diagnostics may be undertaken by isolating genomic and/or proteomic sequences of a pathogen and determining a Replikin concentration of the pathogen. A single specimen with a Replikin concentration greater than 4.0 returns a warning signal that the specimen is from a population of drug-resistant pathogens and more specimens should be analyzed from the population since many populations of bacteria have no Replikin concentrations of 4.0 or greater and all populations of resistant bacteria examined show specimens having Replikin concentrations greater than 4.0. The presence of a specimen having a Replikin concentration of greater than 4.0 raises the risk that the single specimen was obtained from a population of bacteria that is drug-resistant. In a non-limiting embodiment, the drug-resistance of a pathogen is diagnosed where a non-response in a patient suffering from an infection follows administration of an antimicrobial. In another non-limiting embodiment, the drug-resistance of a pathogen is diagnosed where a practitioner observes any reason to believe an antimicrobial treatment is not effective. The method provides a practitioner with evidence of drug-resistance where a Replikin concentration is greater than 4.0 Replikin sequences per 100 amino acid residue and evidence of drug sensitivity where a Replikin concentration is 4.0 or less.

[000108] A non-limiting embodiment of the aspect of the invention provides a method of diagnosing the drug sensitivity or drug resistance of a pathogen comprising (1) determining a

Replikin concentration of a plurality of specimens of an initial population of pathogen at a given time or in a given location and determining the percentage of said specimens having a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues, (2) determining a Replikin concentration of a plurality of specimens of a different population of the pathogen at a different time or different location and determining the percentage of said specimens having a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues, and (3) if the percentage of specimens having a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues in the initial population of pathogens is greater than the percentage of specimens having a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues in the different population, diagnosing the initial population of pathogens as increasing in drug resistance relative to the different population and if the percentage of specimens having a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues in the initial population of pathogens is less than the percentage of specimens having a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues in the different population, diagnosing the initial population of pathogens as decreasing in drug resistance relative to the different population. A population may be considered a drug-resistant population if the percentage of specimens having a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues is 5% or more (see Figure 2), is 10% or more (see Figure 2), is 15% or more (see Figure 3), is 20% or more (see Figures 3 and 4), is 30% or more, is 40% or more, or is 50% or more (see Figure 4).

[000109] The diagnostic method may be practiced on any pathogen including any fungal pathogen, any parasitic pathogen, or any bacterial pathogen. The diagnostic method may be practiced on a malarial pathogen, a Gonococcus pathogen, a Tuberculosis pathogen, a Klebsiella pathogen, a Clostridium pathogen, a Streptococcus pathogen, a Staphylococcus pathogen, or an E. Coli pathogen. In another non-limiting embodiment, the bacterial pathogen is *Neisseria gonorrhoeae*, *Mycobacterium tuberculosis*, *Klebsiella pneumoniae, Clostridium difficile, Streptococcus pneumoniae*, or *Staphylococcus aureus.*

[000110] An increase in percentage of specimens having a Replikin concentration of greater than 4.0 indicates an increase in drug-resistance. Statistical analysis provides levels of certainty concerning differentiation of Replikin concentrations among different populations. P values indicate degree of statistical certainty with reference to the relationship between differences in Replikin concentration and drug-resistance. A p value of less than

0.10 reflects less statistical certainty than a p value of less than 0.05, which reflects less statistical certainty than a p value of less than 0.01, which reflects less statistical certainty than a p value of less than 0.001. Differentiation of Replikin concentrations may be made at p values of less than 0.1, 0.05, 0.01, and 0.001 or whatever statistical device is applied by those of skill in the art. *See, e.g.,* WO 2009/132209, Figures 3 and 4 and description thereof.

Methods of diagnosing drug-resistant pathogens using Replikin sequences

[000111] Another non-limiting aspect provides a method of diagnosing drug-resistant pathogens comprising identifying a Replikin sequence of a drug-resistant pathogen or Replikin Peak Gene sequence of a drug-resistant pathogen in an animal, including human, infected by a drug-resistant pathogen identified by the methods described herein. In a non-limiting embodiment, the blood or tissue of human or other animal may be screened for a Replikin sequence or a Replikin Peak Gene sequence of a drug-resistant pathogen. In a further non-limiting embodiment, an antibody or other binding agent may be used to screen for a Replikin sequence or Replikin Peak Gene sequence. In a further non-limiting embodiment, the blood or tissue of human or other animal may be screened for DNA or RNA that includes a sequence encoding for a Replikin sequence or a Replikin Peak Gene sequence.

[000112] In a further non-limiting embodiment, nucleic acid sequences encoding for (or antisense to) Replikin sequences or Replikin Peak Gene sequences may be used in hybridization assays of biopsied tissue or blood, *e.g.*, Southern or Northern analysis, including in situ hybridization assays, to diagnose the presence of a drug-resistant pathogen in a tissue sample or an environmental sample. A further non-limiting embodiment contemplates kits containing antibodies specific for particular Replikins that are present in a particular pathogen of interest, or containing nucleic acid molecules (sense or antisense) that hybridize specifically to a particular Replikin, and optionally, various buffers and/or reagents needed for diagnosis.

Antibodies and antibody derivatives and passive immunity

[000113] Another aspect of the invention provides binding agents that bind at least to a functional fragment of a Replikin sequence identified in a drug-resistant pathogen. Binding agents are provided including an antibody, antibody fragment, or binding agent that binds to at least a portion of an amino acid sequence of at least one protein, protein fragment, polypeptide, or peptide comprising at least one peptide A, where peptide A is at least 30%, 40%, 50%, 60%, 70%, 80%, 90% or 95%, or 100%, homologous with at least one Replikin peptide identified in a drug-resistant pathogen, which may include, for example, at least one

Replikin peptide sequence of SEQ ID NO(s): 1-119. The amino acid sequence of a protein fragment, polypeptide, or peptide may partially match the amino acid sequence of an expressed whole protein where at least one, five, ten, twenty, thirty, forty, fifty, one hundred, two hundred, three hundred, four hundred, five hundred or more amino acid residues of the amino acid sequence of the expressed whole protein are not present in the protein fragment, polypeptide, or peptide. The amino acid sequence of the protein fragment, polypeptide, or peptide may also partially match the amino acid sequence of an expressed whole protein where at least one, ten, twenty, thirty, forty, fifty, sixty, seventy, eighty, ninety, one hundred, one hundred fifty, two hundred, two hundred fifty, three hundred, three hundred fifty, four hundred, four hundred fifty, five hundred, five hundred fifty or more amino acid residues of the amino acid sequence of at least one terminus of the expressed whole protein are not present at least one terminus of said protein fragment, polypeptide, or peptide.

[000114] Binding agents are also provided including an antibody, antibody fragment, or binding agent that binds to at least a portion of an amino acid sequence that is 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% or more homologous with at least one Replikin peptide of a drug-resistant bacterium. In a non-limiting embodiment, the length of a polypeptide comprising the Replikin peptide sequence or homologue may be one, five, ten, twenty, thirty, forty, fifty or more amino acid residues longer than the identified Replikin sequence with which it is homologous. Binding agents are also provided that bind to at least a portion of an amino acid sequence of at least one of SEQ ID NO(s): 1-119.

[000115] Binding agents may specifically or preferentially bind to the target protein, protein fragment, polypeptide, or peptide. Binding agents may specifically or preferentially bind to a homologue of at least one of SEQ ID NO(s): 1-119. Binding agents may likewise specifically or preferentially bind to a peptide consisting of any one of SEQ ID NO(s): 1-119. Binding agents may also specifically or preferentially bind to a portion of a peptide consisting of any one of SEQ ID NO(s): 1-119 including a single amino acid within a homologue of SEQ ID NO(s): 1-119, two amino acids, three amino acids, four amino acids, five amino acids, or any number of amino acids spread within or outside a homologue.

[000116] In a non-limiting embodiment, the isolated Replikin peptide sequences may be used to generate antibodies, which may be used, for example for diagnostic purposes, to identify protein or protein fragments of interest for development of vaccines and other therapies, or, for example, to provide passive immunity in an subject. Various procedures known in the art may be used for the production of antibodies to Replikin sequences. Such

antibodies include but are not limited to polyclonal, monoclonal, chimeric, humanized, single chain, Fab fragments and fragments produced by a Fab expression library. Antibodies that are linked to a cytotoxic agent may also be generated. Antibodies may also be administered in combination with an antiviral agent. Furthermore, combinations of antibodies to different Replikins may be administered as an antibody cocktail.

[000117] An antibody or other binding agent of an aspect of the invention may bind to a Replikin peptide or a Replikin Peak Gene. It may bind to a protein or protein fragment comprising a Replikin peptide or a Replikin Peak Gene. It may also bind to a portion of a Replikin peptide or a portion of a Replikin Peak Gene or a portion of a protein, protein fragment, polypeptide, or peptide comprising a Replikin peptide or Replikin Peak Gene.

[000118] An antibody or other binding agent that specifically binds to a portion of a Replikin peptide or a portion of a Replikin Peak Gene generally binds to an epitope on the Replikin peptide or an epitope that is at least partially on the Replikin peptide or to an epitope on the Replikin Peak Gene or an epitope that is at least partially on the Replikin Peak Gene when the antibody or fragment of the antibody binds to the epitope more readily than it would bind to a random, unrelated epitope.

[000119] Monoclonal antibodies to Replikin sequences may be prepared by using any technique that provides for the production of antibody molecules. These include but are not limited to the hybridoma technique originally described by Kohler and Milstein, (*Nature*, 1975, 256:495-497), the human B-cell hybridoma technique (Kosbor et al., 1983, *Immunology* Today, 4:72), and the EBV hybridoma technique (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96). In addition, techniques developed for the production of chimeric antibodies (Morrison et al., 1984, *Proc. Nat. Acad. Sci USA*, 81:6851-6855) or other techniques may be used. Alternatively, techniques described for the production of single chain antibodies (U.S. 4,946,778) can be adapted to produce Replikin-specific single chain antibodies.

[000120] Antibodies or other binding agents to any peptides observed to be present in a drug-resistant pathogen and combinations of such antibodies are useful in the treatment and/or prevention of pathogenic infection, including Replikin peptide sequences and functional fragments thereof, Replikin Peak Gene peptide sequences, and Replikin sequences isolated within Replikin Peak Gene peptide sequences.

[000121] Antibody fragments that contain binding sites for a Replikin sequence may be generated by known techniques. For example, such fragments include but are not limited to

F(ab')2 fragments which can be produced by pepsin digestion of the antibody molecules and the Fab fragments that can be generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries can be generated (Huse et al., 1989, *Science*, 246:1275-1281) to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity.

[000122] In another aspect of the invention, immune serum containing antibodies to one or more Replikin sequences obtained from an individual exposed to one or more Replikin sequences may be used to induce passive immunity in another individual or animal. Immune serum may be administered via *i.v.* to a subject in need of treatment. Passive immunity also can be achieved by injecting a recipient with preformed antibodies to one or more Replikin sequences or functional fragments thereof. Passive immunization may be used to provide immediate protection to individuals who have been exposed to a drug-resistant infectious pathogen. Administration of immune serum or preformed antibodies is routine and the skilled practitioner can readily ascertain the amount of serum or antibodies needed to achieve the desired effect. One of the reasons that vaccines directed towards a particular protein antigen of a disease-causing agent have not been fully effective in providing protection against the disease is that the best antibodies have not been produced, that is – it is likely that the antibodies to the Replikin sequences or functional fragment thereof have not been produced.

Anti-Sense Nucleic Acids and siRNA

[000123] The invention further provides a nucleic acid sequence that is antisense to a nucleic acid that encodes for any Replikin peptide present in or identified in a drug-resistant bacterial isolate. This may include one of SEQ ID NO(s): 1-119 or a small interfering nucleic acid sequence that interferes with a nucleic acid sequence that is 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% or more homologous with a nucleic acid that encodes any Replikin peptide sequence of a drug-resistant pathogen including, for example, any one of SEQ ID NO(s): 1-119 or is 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95% or more homologous with a nucleic acid that is antisense to a nucleic acid that encodes for any one of SEQ ID NO(s): 1-119. Antisense and siRNA sequences are short and directed specifically against the sequence that encodes a Replikin sequence or a fragment thereof.

[000124] The nucleotide sequence of the invention may be used in hybridization assays of biopsied tissue or blood, *e.g.*, Southern or Northern analysis, including in situ hybridization assays, to diagnose the presence of a particular drug-resistant bacterial strain in a tissue

sample or an environmental sample, for example. The present invention also provides kits containing antibodies or binding agents specific for particular Replikin sequences or functional fragments thereof that are present in a particular isolate, or containing nucleic acid molecules (sense or antisense) that hybridize specifically to a particular Replikin sequence or fragment thereof, and optionally, various buffers and/or reagents needed for diagnosis.

[000125] Also within the scope of an aspect of the invention are oligoribonucleotide sequences that include antisense RNA and DNA molecules and ribozymes that function to inhibit the translation of Replikin-containing mRNA. Both antisense RNA and DNA molecules and ribozymes may be prepared by any method known in the art. The antisense molecules can be incorporated into a wide variety of vectors for delivery to a subject. The skilled practitioner can readily determine the best route of delivery, although generally intravenous or intramuscular delivery is routine. The dosage amount is also readily ascertainable.

[000126] An aspect of the invention further provides antisense nucleic acid molecules that are complementary to a nucleic acid of the invention, wherein the antisense nucleic acid molecule is complementary to a nucleotide sequence encoding a peptide of the invention. In particular the nucleic acid sequence may be anti-sense to a nucleic acid sequence that has been demonstrated to be conserved over a period of six months to one or more years and/or which are present in a strain of drug-resistant bacteria shown to have an increase in concentration of Replikin sequences.

[000127] An aspect of the invention also provides compositions comprising RNAi-inducing entities used to inhibit or reduce bacterial infection or replication including small interfering RNA, which is a class of about 10 to about 50 and often about 20 to about 25 nucleotide-long double-stranded RNA molecules. siRNA is involved in the RNA interference pathway, where it interferes with the expression of a specific gene of the bacterium. siRNAs also act in RNAi-related pathways, *e.g.*, as an antimicrobial mechanism.

[000128] An effective amount of an RNAi-inducing entity is delivered to a subject prior to or at the time of infection. A dosage may be sufficient to reduce or delay one or more symptoms of infection. Compositions of the invention may comprise a single siRNA species targeted to a target transcript or may comprise a plurality of different siRNA species targeting one or more target transcripts.

[000129] The invention provides a small interfering nucleic acid sequence that is about 10 to about 50 nucleic acids in length and is 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% or

more homologous with a nucleic acid that encodes for any portion of a bacterial Replikin peptide including, for example, any portion of SEQ ID NO(s): 1-119 or is 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 95% or more homologous with a nucleic acid that is antisense to a nucleic acid that encodes for any portion of a Replikin peptide, including, for example, a portion of one of SEQ ID NO(s): 1-119. In a further non-limiting embodiment, the nucleic acid sequence is about 15 to about 30 nucleic acids. In a further non-limiting embodiment, the nucleic acid sequence is about 20 to about 25 nucleic acids. In a further non-limiting embodiment, the nucleic acid sequence is about 21 nucleic acids.

Computer Methods for Determining Drug Resistance

[000130] A determination of a drug-resistant pathogen or population of pathogens may be performed by a processor. A determination may be output to a user or display. Likewise, a particular Replikin peptide or Replikin Peak Gene within an isolate or population of isolates of one or more drug-resistant pathogens or pathogens identified as increasing in drug-resistance may be identified and output to a user or display. A machine-readable storage medium may contain executable instructions that, when executed by a processor, cause the processor to provide sufficient data to a user, a printout, or a display such that the user or a user of the printout or display may identify drug resistance in a pathogen or population of pathogen. A non-limiting computer readable medium may be non-transitory. Software comprising methods of the invention and related data may be carried on a signal.

[000131] A computer system may include a processor coupled to a network, and a memory coupled to a processor, wherein the memory contains a plurality of instruction to perform the methods of determining drug-resistance described herein. A user of outputted data from a processor, storage medium, machine-readable medium, or computer system may include any person or any machine that records or analyzes the outputted data. A display or printout may include any mechanism by which data is outputted so that any person or any machine may record or analyze the outputted data, including a printed document, a visual impulse, an aural impulse, or any other perceivable impulse, a computer monitor, a set of numbers, or any other display or printout of data including a digital recording medium.

Example 1

Analysis of Replikin concentration above 4.0 in Drug-sensitive and Drug-resistant Specimens of Gonococcus

[000132] Applicants queried the PubMed website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) for reported sequences from Gonococcus (*Neisseria gonorrhoeae*) specimens reported as generally sensitive to antibiotics or as resistant to specific antibiotics (Cefixime, Rifampicin, Quinoline, and Tetracycline). Replikin concentration was determined for specimens identified as drug sensitive, specimens identified as resistant to the Cefixime antibiotic, specimens identified as resistant to Rifampicin antibiotic, specimens identified as resistant to Quinoline antibiotic, and specimens identified as resistant to Tetracycline antibiotic. Percentage of specimens in a particular group having a Replikin concentration of greater than 4.0 was recorded. The percentage of specimens with Replikin concentrations of greater than 4.0 and the percentage of bacteria resistant to a particular antibiotic were observed to be proportional to the time in use of the particular antibiotic.

[000133] Table 1 provides the percentage of specimens of Gonococcus in a given drug-sensitive or drug-resistant population having a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues. The data reflect analysis of 5,110 genomic sequences of Gonococcus. The data are illustrated in Figure 1.

TABLE 1
(n=5,110)

Sensitivity or Resistance	Percent Specimens with Replikin Concentration Greater than 4.0
Drug Sensitive	3
Cefixime Resistant	54
Rifampicin Resistant	65
Quinoline Resistant	86
Tetracycline Resistant	95

[000134] Among Gonococcus populations, the percent of specimens with a Replikin concentration above 4.0 and the percent of resistant bacteria both are observed to be proportional to the amount of time that a given class of antibiotics has been in use.

Example 2

Analysis of Replikin Concentration above 4.0 in Specimens of Specific Drug-sensitive, Drug-resistant, Attenuated Virulence, and Virulent Strains of *Mycobacterium tuberculosis*

[000135] Applicants queried the PubMed website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) for reported sequences from tuberculosis pathogens (*Mycobacterium tuberculosis*) of five specific strains. Strain KZN 4207 was identified as fully drug sensitive, strain KZN 1435 was identified as drug resistant, strain KZN R506 was identified as drug resistant, and strain KZN 605 was identified as extremely drug resistant. Strains H37Ra and H37Rv were identified as attenuated virulence and virulent, respectively. Number of sequences analyzed for each of strains KZN 4207, KZN 1435, KZN R506, H37Ra, H37Rv, and KZN 605 were 3,883, 2,610, 1,278, 3,350, 1,217, and 2,592, respectively. Percentage of sequences having a concentration of greater than 4.0 are individually illustrated in Figure 2 for each strain.

[000136] Analyses revealed that of each population of tuberculosis specimen provided Replikin concentrations in the range from 0.1 to 4 for drug sensitive populations and over 4.0 to 10.0 for more drug-resistant populations with occasional sequences having Replikin concentrations of 20 to 30. The percent of specimens of these tuberculosis populations that contain Replikin concentrations greater than 4.0 % is seen to increase from 1.9% for sequences from 'Fully Drug Sensitive' KZN 4027 specimens, to 5.7 and 5.8 in Drug Resistant specimens, KZN 1435, KZN R506, to 6.3 in the 'avirulent' strain H37Ra and 6.7 in the 'virulent' strain H37Rv, to 12.7 in the "Extremely Drug Resistant" tuberculosis KZN 605. The percentage of 12.7% of specimens with Replikin concentrations of greater than 4.0 in the "Extremely Drug Resistant" tuberculosis KZN 605 is seen to be six-fold greater than that of the 'Fully Drug Sensitive' genomes.

[000137] Genomic Replikin concentration in influenza virus has previously been shown to range from 0.1 to 4.0 for viruses not rapidly replicating and not producing outbreaks to over 4.0 to 10.0 or more for outbreaks. Minorities of viruses occasionally are observed to have Replikin concentrations of 20 to 30.

[000138] Table 2 provides the percentage of specimens of specific strains of tuberculosis with particular drug sensitivities or virulence having a Replikin concentration of greater than 4.0. The data are illustrated in Figure 2.

TABLE 2

Relative Drug Sensitivity or Virulence	Strain	Percent of Specimens with Replikin Concentration Greater than 4.0	Number of Sequences Analyzed
Fully Drug Sensitive	KZN4207	1.9	3,883
Drug Resistant	KZN1435	5.7	2,610
Drug Resistant	KZNR506	5.8	1,278
Attenuated Virulence	H37Ra	6.3	3,350
Virulent	H37Rv	6.7	1,217
Extremely Drug Resistant	KZN605	12.7	2,592

[000139] A vaccine is provided herein against drug-resistant, highly-lethal tuberculosis strains. The information also now provides up to two years of time to thoroughly test and distribute vaccines to high risk individuals in the countries identified; thus for the first time, a quantitative genomic Replikins method to both predict initial outbreaks and to prevent spread.

[000140] The vaccine comprises Replikin sequences identified in drug-resistant strains of tuberculosis. One vaccine comprises Replikin sequences identified in the extremely drug-resistant KZN 605 strain.

[000141] Tuberculosis remains a major infectious disease, which accounts for approximately 3 million deaths per year worldwide. A series of effective drugs have been developed over several decades which have been recently challenged by two new circumstances: the first is the mutation of the tuberculosis genome to more lethal drug resistant forms, and the second is the association of tuberculosis with AIDS, both resulting in high mortality cases in such areas as KwaZulu-Natal in South Africa and in Europe and Asia. In Figure 2, populations of pathogen isolated from KwaZulu-Natal are denoted with the prefix KZN.

Example 3

Analysis of Replikin concentration above 4.0 in carbapenem-resistant specimens of *Klebsiella pneumoniae* from 2007 through 2012

[000142] Applicants queried the PubMed website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) for reported sequences from specimens of carbapenem-resistant *Klebsiella pneumoniae* and identified those sequences having a Replikin concentration of greater than 4.0 per 100 amino acid residues for specimens isolated in years 2007 through 2012. Replikin sequences identified from 2001 through 2012 are reported in Example 7 herein.

[000143] Table 3 provides the percentage of specimens of carbapenem-resistant Klebsiella in a given year having a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues. The data are illustrated in Figure 3. As in all analyses of Replikin concentrations in sequences published at the PubMed website, not all sequences are timely published. As a result, not all sequences from the immediate previous quarter of the year are available. At present, concern about outbreaks of carbapenem-resistant Klebsiella has recently been expressed by the CDC (in, for example, the New York Times and elsewhere) but neither current clinical nor current sequence data has yet been published. Nevertheless, the data concerning percentage of specimens of Klebsiella having Replikin concentrations of greater than 4.0 suggest the 2011 outbreak of Klebsiella is not yet over.

TABLE 3

Year	Percent Specimens with Replikin Concentration Greater Than 4.0
2007	0%
2008	6.7%
2009	15.7%
2010	22.1%
2011	20.1%
2012	28.9%

[000144] *Klebsiella pneumoniae* is reported as a significant cause of nosocomial infections. Infections are particularly reported in patients with compromised immunities. An

outbreak of carbapenem-resistant *Klebsiella pneumoniae* struck the U.S. National Institutes of Health Clinical Center in 2011 affecting 18 patients. Eleven of the 18 patients died of the infection.

[000145] Snitkin *et al.* reported whole genome sequences of bacterial isolates from infected patients in an attempt to identify and track the course of the infection. Sci Transl Med 22 August 2012: Vol. 4, Issue 148, p. 148ra116. The authors contend that integrated genomic and epidemiological analysis traced the outbreak to three independent transmissions from a single patient. The patient left the hospital three weeks before a follow-on infection became clinically identifiable. *Id.* No methods or structures for advance prediction or targets for design of therapies or vaccines were provided.

[000146] Prior to the current invention, no structural genomic information was available to determine association with virulence and Replikin concentration. Figure 3 illustrates that an increase in percentage of specimens of carbapenem-resistant *Klebsiella pneumoniae* with Replikin concentration greater than 4.0 rose to 22% in the year before the outbreak and maintained a level of 20% at the time of the outbreak. Replikin sequences for manufacture of a vaccine again an outbreak of carbapenem-resistant *Klebsiella pneumoniae* are provided in Example 7.

Example 4

Analysis of Replikin concentration above 4.0 in drug-resistant Specimens of *Clostridium difficile* from 1998 through 2011

[000147] Applicants queried the PubMed website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) for reported sequences from specimens of drug-resistant *Clostridium difficile.* Specimens were available for years 1998 through 2011. Applicants analyzed the sequences and identified the percentage of sequences having a Replikin concentration of greater than 4.0 per 100 amino acid residues for specimens isolated in years 1998 through 2011.

[000148] Table 4 provides the percentage of specimens of drug-resistant *Clostridium difficile* reported as isolated in years 1998 through 2011 and having a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues. The data reflect analysis of 86,581 genomic sequences of *Clostridium difficile.* The data are illustrated in Figure 4.

TABLE 4
(n=86,581)

Year	Percent Specimens with Replikin Concentration Greater Than 4.0
1998	46.1
1999	53.3
2000	25.0
2001	33.3
2002	66.7
2003	37.5
2004	33.3
2005	25.0
2006	46.3
2007	46.5
2008	47.0
2009	45.2
2010	45.8
2011	45.8

[000149] From the identified Replikin sequences in specimens of *Clostridium difficile*, the Applicants designed a vaccine against the drug-resistant pathogen. The vaccine is described in Example 5 below.

Example 5

Vaccine against drug-resistant *Clostridium difficile*

[000150] A vaccine against drug-resistant *Clostridium difficile* was developed. The vaccine comprises any one or more of the following sequences:

KMKTHRGAAK (SEQ ID NO: 1)

KTHRGAAK (SEQ ID NO: 2)

HRGAAKRLKKTGTGK (SEQ ID NO: 3)

KRAKAFKKH (SEQ ID NO: 4)

KKHILTK (SEQ ID NO: 5)

KHILTKK (SEQ ID NO: 6)

HILTKKSAKTK (SEQ ID NO: 7)

KKYKRAKH (SEQ ID NO: 8)

KYKRAKHMK (SEQ ID NO: 9)

KRAKHMK (SEQ ID NO: 10)

KHMKDNWK (SEQ ID NO: 11)

HMKDNWKTK (SEQ ID NO: 12)

KNGNCTGVLKNDSPH (SEQ ID NO: 13)

KNGGYVEGKH (SEQ ID NO: 14)

HQRVKIDKRIEK (SEQ ID NO: 15)

HYHRSNKGGGQWEHK (SEQ ID NO: 16)

HRSNKGGGQWEHKK (SEQ ID NO: 17)

KGGGQWEHK (SEQ ID NO: 18)

KNLKFHIK (SEQ ID NO: 19)

KFHIKPTGFK (SEQ ID NO: 20), and

HVDAAKGMVNWAK (SEQ ID NO: 21).

[000151] The vaccine targets replication of drug-resistant *Clostridium difficile* by targeting the structure and function of Replikin sequences. The immunogenic nature of Replikin sequences allows for an immune response. A blocking response is also provided.

Example 6

Vaccine against drug-resistant Streptococcus species

[000152] A vaccine against drug-resistant Streptococcus species was developed. The vaccine comprises any one or more of the following sequences:

KFATATLAIQKGAH (SEQ ID NO: 22)

KPNAIIMDKAVEH (SEQ ID NO: 23)

HMSKKMLKIK (SEQ ID NO: 24)

KIEHKDCSK (SEQ ID NO: 25)

KTGELNHQVK (SEQ ID NO: 26)

HQVKGTKPRK (SEQ ID NO: 27)

HYALKAMGYTRK (SEQ ID NO: 28)

KAMGYTRKKEPH (SEQ ID NO: 29)

KQKTHRASAK (SEQ ID NO: 30)

KTHRASAKRFK (SEQ ID NO: 31)
KTHRASAK (SEQ ID NO: 32)
KKQRRHLRK (SEQ ID NO: 33)
KASMVHSGDYK (SEQ ID NO: 34)
KAPQTIHNEVK (SEQ ID NO: 35)
HNEVKRGTTLQQVRK (SEQ ID NO: 36)
KKLILTKEIREKILH (SEQ ID NO: 37)
KILHYHK (SEQ ID NO: 38)
HKQKFSPEMMVNK (SEQ ID NO: 39)
HNGHLGLTKADMLYPRK (SEQ ID NO: 40)
HLGLTKADMLYPRK (SEQ ID NO: 41)
HNRLIRRWLPKGTKKTTPK (SEQ ID NO: 42)
KSFCRNHK (SEQ ID NO: 43)
HIVRTIYCKECGKELIQK (SEQ ID NO: 44)
KECGKELIQKEH (SEQ ID NO: 45)
KELIQKEHTK (SEQ ID NO: 46)
KEHTKKK (SEQ ID NO: 47)
HTKKKIFCSDACK (SEQ ID NO: 48)
KKIFCSDACKRKWWNGH (SEQ ID NO: 49)
KRKWWNGHRK (SEQ ID NO: 50)
KIFCSDACKRKWWNGH (SEQ ID NO: 51)
KWWNGHRK (SEQ ID NO: 52)
HKKFKAYVAEHRK (SEQ ID NO: 53)
KFKAYVAEHRK (SEQ ID NO: 54)
KAYVAEHRK (SEQ ID NO: 55), and
KYCCHSCYIK (SEQ ID NO: 56).

[000153] The vaccine targets replication of drug-resistant Streptococcus species by targeting the structure and function of Replikin sequences. The immunogenic nature of Replikin sequences allows for an immune response. A blocking response is also provided.

Example 7

Replikin sequences identified as conserved in sequences reported for carbapenem-resistant specimens of *Klebsiella pneumoniae* from 2007 through 2012

[000154] Applicants queried the PubMed website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/) for *Klebsiella pneumoniae* specimens reported as isolated in 2002 through 2012. The following sequences were identified as conserved. Accession numbers in which the sequences were identified and are reported below along with the year provided in the accession number. Replikin concentrations for specimens in each year for Klebsiella were determined. The Replikin concentration for specimens in year 2012 was 27.1.

KALALITKISPAAWRH (SEQ ID NO: 57)

[000155] All occurrences of the sequence by year:

2002	AAM10640 position 379
2008	ABZ10934 position 966 , ABZ10928 position 966 , ABZ10922 position 966 , ABZ10916 position 966 .
2010	YP_003560390 position 966 , ADE43960 position 966 .
2011	AEV55252 position 966 , AEI25490 position 966 .
2012	AFV66807 position 966 , ZP_18999014 position 165 , ZP_18999013 position 29 , YP_006971130 position 966 , YP_006958858 position 966 , YP_006958632 position 966 .

KIDCDLIHREK (SEQ ID NO: 58)

[000156] All occurrences of the sequence by year:

2002	AAM10640 position 237 .
2008	ABZ10934 position 824 , ABZ10928 position 824 , ABZ10922 position 824 , ABZ10916 position 824 .
2010	YP_003560390 position 824 , ADE43960 position 824 .
2011	AEV55252 position 824 .
2012	AFV66807 position 824 , ZP_18999014 position 23 , ZP_18999015 position 305 , YP_006971130 position 824 , YP_006958858 position 824 .

KTHLAIALGYK (SEQ ID NO: 59)

[000157] All occurrences of the sequence by year:

2002	AAM10642 position 114 .
2003	AAO53444 position 15 .
2006	ABF81677 position 106 .
2007	ABJ91197 position 114 , ABJ91194 position 114 , CAO82095 position 114 .

2008	ABZ10936 position 114 , ABZ10930 position 114 , ABZ10924 position 114 , ABZ10918 position 114 .
2010	YP_003560392 position 114 , ADE43962 position 114 .
2011	AEV55250 position 114 , AEI25492 position 114 .
2012	YP_006964673 position 114 , AFM72617 position 114 , AFV66809 position 114 , YP_006971132 position 114 , YP_006958856 position 114 , YP_006958634 position 114 .

HCSSREGKESTEMK (SEQ ID NO: 60)

[000158] All occurrences of the sequence by year:

2000	NP_071347 position 460 .
2004	AAU93470 position 460 .
2006	Q5XPI4 position 460

HLRKTLKDDLASK (SEQ ID NO: 61)

[000159] All occurrences of the sequence by year:

2000	NP_071347 position 611 .
2004	NP_115932 position 611 , AAU93471 position 611 , AAU93470 position 611 .
2006	Q5XPI3 position 611 , Q5XPI4 position 611 .
2010	D3ZXK7 position 609 .

HLRKTLKDDLASK (SEQ ID NO: 62)

[000160] All occurrences of the sequence by year:

2000	NP_071347 position 611 .
2004	NP_115932 position 611 , AAU93471 position 611 , AAU93470 position 611 .
2006	Q5XPI3 position 611 , Q5XPI4 position 611 .
2010	D3ZXK7 position 609 .

HLMNNKDCFFCK (SEQ ID NO: 63)

[000161] All occurrences of the sequence by year:

2000	NP_071347 position 1281 .
2004	NP_115932 position 1281 , AAU93471 position 1281 , AAU93470 position 1281 .
2006	Q5XPI3 position 1281 , Q5XPI4 position 1281 .
2010	D3ZXK7 position 1285 .

KANASRHK (SEQ ID NO: 64)

[000162] All occurrences of the sequence by year:

2006	ABF81679 position 147 .
2007	ABJ91199 position 147 , ABJ91196 position 147 .
2008	ABZ10938 position 147 , ABZ10932 position 147 , ABZ10926 position 147 , ABZ10920 position 147 .
2010	YP_003560394 position 147 , ADE43964 position 147 .
2011	AEV55248 position 147 , AEI25494 position 147 .
2012	YP_006964675 position 147 , AFM72619 position 147 , AFV66811 position 147 , ZP_19000196 position 147 , YP_006971134 position 147 , YP_006958854 position 147 , YP_006958636 position 147 .

HKAMSYKRMK (SEQ ID NO: 65)

[000163] All occurrences of the sequence by year:

2006	ABF81679 position 153 .
2007	ABJ91199 position 153 , ABJ91196 position 153 .
2008	ABZ10938 position 153 , ABZ10932 position 153 , ABZ10926 position 153 , ABZ10920 position 153 .
2009	ACS75443 position 40 .
2010	YP_003754011 position 40 , YP_003560394 position 153 , ADE43964 position 153 .
2011	AEV55248 position 153 , AEI54172 position 40 , AEI25494 position 153
2012	YP_006964675 position 153 , AFM72619 position 153 , AFV66811 position 153 , ZP_19000196 position 153 , YP_006971134 position 153 , YP_006958854 position 153 , YP_006958636 position 153 .

HCEIKALLDRAK (SEQ ID NO: 66)

[000164] All occurrences of the sequence by year:

2006	ABF81679 position 169 .
2007	ABJ91199 position 169 , ABJ91196 position 169 .
2008	ABZ10938 position 169 , ABZ10932 position 169 , ABZ10926 position 169 , ABZ10920 position 169 .
2009	ACS75443 position 56 .
2010	YP_003754011 position 56 , YP_003560394 position 169 , ADE43964 position 169 .
2011	AEV55248 position 169 , AEI54172 position 56 , AEI25494 position 169 .
2012	YP_006964675 position 169 , AFM72619 position 169 , AFV66811 position 169 , ZP_19000196 position 169 , YP_006971134 position 169 , YP_006958854 position 169 , YP_006958636 position 169 .

HPDGSDKGGGSYK (SEQ ID NO: 67)

[000165] All occurrences of the sequence by year:

2006	ABF81679 position 238 .
2007	ABJ91199 position 238 , ABJ91196 position 238 .
2008	ABZ10938 position 238 , ABZ10932 position 238 , ABZ10926 position 238 ,

	ABZ10920 position 238 .
2009	ACM47590 position 62 , ACS75443 position 125 .
2010	YP_003754011 position 125 , YP_003560394 position 238 , ADE43964 position 238 .
2011	AEV55248 position 238 , AEN02812 position 68 , AEN02809 position 68 , AEI54172 position 125 , AEI25494 position 238 .
2012	YP_006964675 position 238 , AFM72619 position 238 , AFV66811 position 238 , ZP_19000196 position 238 , YP_006971134 position 238 , YP_006958854 position 238 , YP_006958636 position 238 .

KSHAPNK (SEQ ID NO: 68)

[000166] All occurrences of the sequence by year:

2007	ABK57117 position 21 .
2009	ACM47587 position 21 , ACS75441 position 52 .
2010	YP_003754013 position 52 .
2011	AEN02801 position 52 , AEN02791 position 52 , AEI54175 position 52 .

HKTPVQALK (SEQ ID NO: 69)

[000167] All occurrences of the sequence by year:

2007	ABK57117 position 265 .
2009	ACM47587 position 265 , ACS75441 position 296 .
2010	YP_003754013 position 296 .
2011	AEN02810 position 81 , AEN02807 position 81 , AEN02801 position 296 , AEN02791 position 296 , AEI54175 position 296 .

KTPVQALKEWH (SEQ ID NO: 70)

[000168] All occurrences of the sequence by year:

2007	ABK57117 position 266 .
2009	ACM47587 position 266 , ACS75441 position 297 .
2010	YP_003754013 position 297 .
2011	AEN02810 position 82 , AEN02807 position 82 , AEN02801 position 297 , AEN02791 position 297 , AEI54175 position 297 .

HEKRPELFRK (SEQ ID NO: 71)

[000169] All occurrences of the sequence by year:

2007	ABK57117 position 276 .
2009	ACM47587 position 276 , ACS75441 position 307 .
2010	YP_003754013 position 307 .
2011	AEN02810 position 92 , AEN02807 position 92 , AEN02801 position 307 , AEN02791 position 307 , AEI54175 position 307 .

KASWVHK (SEQ ID NO: 72)

[000170] All occurrences of the sequence by year:

2007	ABO09820 position 329

KRNRHAQK (SEQ ID NO: 73)

All occurrences of the sequence by year:

2008	ABZ10912 position 59 .
2010	YP_003560385 position 59 , ADE43955 position 59 .
2011	AEI25498 position 59 .
2012	YP_006958627 position 59 .

HGIKARLPEK (SEQ ID NO: 74)

[000171] All occurrences of the sequence by year:

2008	ABZ10913 position 68 .
2010	YP_003560386 position 68 , ADE43956 position 68 .
2011	AEI25486 position 68 .
2012	YP_006958628 position 68 .

HLVGKGGKPGK (SEQ ID NO: 75)

[000172] All occurrences of the sequence by year:

2008	ABZ10933 position 418 , ABZ10927 position 418 , ABZ10921 position 418 , ABZ10915 position 418 .
2010	YP_003560389 position 418 , ADE43959 position 418 .
2011	AEV55253 position 418 , AEI25489 position 418 .
2012	AFV66857 position 418 , ZP_18996962 position 319 , YP_006971180 position 418 , YP_006958859 position 418 , YP_006958631 position 418 .

KLTHLEYNK (SEQ ID NO: 76)

[000173] All occurrences of the sequence by year:

2008	ABZ10934 position 555 , ABZ10928 position 555 , ABZ10922 position 555 , ABZ10916 position 555 .
2010	YP_003560390 position 555 , ADE43960 position 555 .
2011	AEV55252 position 555 , AEI25490 position 555 .
2012	AFV66807 position 555 , ZP_18999015 position 36 , YP_006971130 position 555 , YP_006958858 position 555 , YP_006958632 position 555 .

HWEKPATLNTDK (SEQ ID NO: 77)

[000174] All occurrences of the sequence by year:

2008	ACJ61269 position 2 .
2010	YP_003675800 position 137 , ADH29558 position 137 .

2011	AEV55232 position 137 .

HGKLKILIK (SEQ ID NO: 78)

[000175] All occurrences of the sequence by year:

2008	ACJ61269 position 50 .
2010	YP_003675800 position 185 , ADH29558 position 185 .
2011	AEV55232 position 185 .

KLDRLGRSLKH (SEQ ID NO: 79)

[000176] All occurrences of the sequence by year:

2008	ACJ61270 position 62 .

KLDLYGKIDGLH (SEQ ID NO: 80)

[000177] All occurrences of the sequence by year:

2009	ACM07444 position 31 , ACM07445 position 31 , ACM07443 position 31 .
2012	ZP_18998071 position 31 .

KRLVINKEKH (SEQ ID NO: 81)

[000178] All occurrences of the sequence by year:

2009	ACS75439 position 445 .
2010	YP_003754015 position 445 .
2011	AEW43364 position 445 .
2012	YP_006959187 position 445 .

KGHLRPLK (SEQ ID NO: 82)

[000179] All occurrences of the sequence by year:

2009	ACS75439 position 985 .
2010	YP_003754015 position 985 .
2011	AEW43364 position 985 , AEW43350 position 161 .
2012	AFV66855 position 161 , ZP_19000870 position 124 , ZP_18999050 position 161 , YP_006971178 position 161 , YP_006959187 position 985 , YP_006959008 position 161 .

KEPEYEAAKH (SEQ ID NO: 83)

[000180] All occurrences of the sequence by year:

2009	ACS75443 position 28 .
2010	YP_003754011 position 28
2011	AEI54172 position 28 .

KLFSGAKH (SEQ ID NO: 84)

[000181] All occurrences of the sequence by year:

2009	ACS75450 position 303 .
2010	YP_003754005 position 303 .

KHQQQFQK (SEQ ID NO: 85)

[000182] All occurrences of the sequence by year:

2009	ACS75450 position 311 .
2010	YP_003754005 position 311 .

HLKDGAEAK (SEQ ID NO: 86)

[000183] All occurrences of the sequence by year:

2009	ACS75450 position 597 .
2010	YP_003754005 position 597 .

HDRILVKLFSGAK (SEQ ID NO: 87)

[000184] All occurrences of the sequence by year:

2009	ACS75450 position 297 .
2010	YP_003754005 position 297 .

KRSIYFHK (SEQ ID NO: 88)

[000185] All occurrences of the sequence by year:

1997	ADA67982 position 185 .

HLTDKVQSLSK (SEQ ID NO: 89)

[000186] All occurrences of the sequence by year:

2010	YP_003754136 position 135 , YP_003560378 position 135 , ADJ18710 position 135 , ADE43949 position 135 .
2011	AEV55259 position 137 .
2012	YP_006958868 position 137 .

KHSEHKEK (SEQ ID NO: 90)

[000187] All occurrences of the sequence by year:

2010	YP_003560380 position 207 , ADE43951 position 207 .
2011	AEV55258 position 250 .
2012	YP_006958866 position 250 .

KKRATHK (SEQ ID NO: 91)

[000188] All occurrences of the sequence by year:

2010	YP_003560383 position 34 , ADE43953 position 34 .
2011	AEV55337 position 55 .
2012	YP_006958863 position 55 .

KRATHKEVK (SEQ ID NO: 92)

[000189] All occurrences of the sequence by year:

2010	YP_003560383 position 35 , ADE43953 position 35 .
2011	AEV55337 position 56 .
2012	YP_006958863 position 56 .

KWVKNHEK (SEQ ID NO: 93)

[000190] All occurrences of the sequence by year:

2010	YP_003560396 position 111 , ADE43966 position 111 .
2011	AEI25496 position 111 .
2012	YP_006958638 position 111 .

KWNRHLRAQEK (SEQ ID NO: 94)

[000191] All occurrences of the sequence by year:

2010	YP_003560398 position 437 , ADE43968 position 437 .
2011	AEV55244 position 437 .
2012	YP_006964681 position 437 , AFM72625 position 437 , YP_006958850 position 437 .

KTWREAKRQH (SEQ ID NO: 95)

[000192] All occurrences of the sequence by year:

2010	YP_003560398 position 303 , ADE43968 position 303 .
2011	AEV55244 position 303 .
2012	YP_006964681 position 303 , AFM72625 position 303 , YP_006958850 position 303 .

KGEAHHALK (SEQ ID NO: 96)

[000193] All occurrences of the sequence by year:

2010	YP_003560398 position 872 , ADE43968 position 872 .
2011	AEV55244 position 872 .
2012	YP_006964681 position 872 , AFM72625 position 872 , YP_006958850 position 872 .

HLKCCPITTLK (SEQ ID NO: 97)

[000194] All occurrences of the sequence by year:

2010	YP_003754056 position 172 , YP_003560403 position 172 , ADJ18630 position 172 , ADE43973 position 172 .
2011	AEV55332 position 172 .
2012	YP_006958952 position 172 .

KRHLLALK (SEQ ID NO: 98)

[000195] All occurrences of the sequence by year:

2010	YP_003754099 position 61 , YP_003560459 position 61 , ADJ18673 position 61 , ADE44029 position 61 .
2011	AEV55290 position 61 .
2012	ZP_18999970 position 5 , YP_006958906 position 61 .

KDRQDSIHK (SEQ ID NO: 99)

[000196] All occurrences of the sequence by year:

2010	YP_003754128 position 873 , ADJ18702 position 873 .

KHIARSEK (SEQ ID NO: 100)

[000197] All occurrences of the sequence by year:

2010	YP_003560445 position 198 , ADE44015 position 198 .
2011	AEV55156 position 198 .

KWQQHELK (SEQ ID NO: 101)

[000198] All occurrences of the sequence by year:

2012	YP_006971166 position 46 , YP_006958997 position 4

KSAFAHK (SEQ ID NO: 102)

[000199] All occurrences of the sequence by year:

2012	YP_006971154 position 21 , YP_006958987 position 21 .

HKVKNGGK (SEQ ID NO: 103)

[000200] All occurrences of the sequence by year:

2012	YP_006971154 position 26 , YP_006958987 position 26 .

KGDEIQEAKEH (SEQ ID NO: 104)

[000201] All occurrences of the sequence by year:

2012	YP_006971154 position 90 , YP_006958987 position 90 .

KMKGYDVKATH (SEQ ID NO: 105)

[000202] All occurrences of the sequence by year:

2012	YP_006971154 position 205 , YP_006958987 position 205 .

KGYDVKATHK (SEQ ID NO: 106)

[000203] All occurrences of the sequence by year:

2012	YP_006971154 position 207 , YP_006958987 position 207 .

HKQQHGLNQSIK (SEQ ID NO: 107)

[000204] All occurrences of the sequence by year:

2012	YP_006971154 position 215 , YP_006958987 position 215

KQQHGLNQSIK (SEQ ID NO: 108)

[000205] All occurrences of the sequence by year:

2012	YP_006971154 position 216 , YP_006958987 position 216 .

KDAHKTAPK (109)

[000206] All occurrences of the sequence by year:

2012	YP_006971154 position 226 , YP_006958987 position 226 .

HKTAPKRQK (SEQ ID NO: 110)

[000207] All occurrences of the sequence by year:

2012	YP_006971154 position 229 , YP_006958987 position 229 .

KPKQHFIK (SEQ ID NO: 111)

[000208] All occurrences of the sequence by year:

2012	YP_006971154 position 256 , YP_006958987 position 256

HFIKLKTLNK (SEQ ID NO: 112)

[000209] All occurrences of the sequence by year:

2012	YP_006971154 position 260 , YP_006958987 position 260 .

KNGVQHGWK (SEQ ID NO: 113)

[000210] All occurrences of the sequence by year:

2012	YP_006971154 position 312 , YP_006958987 position 312 .

HVDDATKKVAECK (SEQ ID NO: 114)

[000211] All occurrences of the sequence by year:

2012	YP_006971160.1 position 31

KHVDYKDAFK (SEQ ID NO: 115)

[000212] All occurrences of the sequence by year:

2012	YP_006971160 position 68 . YP_006971160 position 31

KEGLFRYKQLH (SEQ ID NO: 116)

[000213] All occurrences of the sequence by year:

2012	YP_006971160 position 56 .

KGKLHVISK (SEQ ID NO: 117)

[000214] All occurrences of the sequence by year:

2010	YP_003560408 position 69 , YP_003754066 position 69 .
2012	YP_006958947 position 69 .

HHARKERIMTK (SEQ ID NO: 118)

[000215] All occurrences of the sequence by year:

2012	YP_006959184 position 46 .

HARKERIMTK (SEQ ID NO: 119)

[000216] All occurrences of the sequence by year:

2012	YP_006959184 position 47 .

Example 8

Vaccine against carbapenem-resistant *Klebsiella pneumoniae*

[000217] A vaccine against carbapenem-resistant *Klebsiella pneumoniae* was developed. The vaccine comprises any one or more of SEQ ID NO(s): 57-119. Each of these peptides is conserved in carbapenem-resistant pathogen and may be comprised in a vaccine that targets carbapenem-resistant *Klebsiella pneumoniae* or *Klebsiella pneumoniae* generally. Any combination of two or more of the sequences may be comprised in such a vaccine. The vaccine may comprise any one of SEQ ID NO(s): 57-119 with the addition of at least one other different sequence from SEQ ID NO(s): 57-119. The vaccine may comprise any combination of two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or any number up to all 63 of the peptides of SEQ ID NO(s): 57-119.

[000218] The vaccine targets replication of drug-resistant Klebsiella species by targeting the structure and function of Replikin sequences. The immunogenic nature of Replikin sequences allows for an immune response. A blocking response is also provided.

What is claimed is:

1. A method of determining if a pathogen is drug sensitive or drug resistant comprising determining a Replikin concentration of said pathogen and diagnosing the pathogen as drug resistant if the Replikin concentration is greater than 4.0 per 100 amino acid residues and drug sensitive if the Replikin concentration is 4.0 per 100 amino acid residues or less.

2. The method of claim 1, wherein said pathogen is a bacterial pathogen.

3. The method of claim 1, wherein said pathogen is a Gonococcus pathogen, a Tuberculosis pathogen, a Klebsiella pathogen, a Clostridium pathogen, a Streptococcus pathogen, a Staphylococcus pathogen, or an E. coli pathogen.

4. An isolated or synthesized protein fragment or peptide comprising at least one peptide sequence that is at least 80% homologous with at least one Replikin peptide sequence identified in a pathogen identified in a population of pathogens having a higher percentage of specimens with a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues than specimens of another population of the same pathogen.

5. An isolated or synthesized protein fragment or peptide comprising at least one functional fragment of at least one Replikin peptide sequence identified in a pathogen identified in a population of pathogens having a higher percentage of specimens with a Replikin concentration of greater than 4.0 Replikin sequences per 100 amino acid residues than specimens of another population of the same pathogen.

6. The isolated or synthesized protein fragment or peptide of claim 4, wherein said population of pathogen is a bacterial pathogen, fungal pathogen, or parasitic pathogen.

7. The isolated or synthesized protein fragment or peptide of claim 4, wherein said population of pathogen is a Gonococcus pathogen, Tuberculosis pathogen, Klebsiella pathogen, Clostridium pathogen, Streptococcus pathogen, Staphylococcus pathogen.

8. The isolated or synthesized protein fragment or peptide of claim 4, wherein said population of pathogen is *Neisseria gonorrhoeae*, *Mycobacterium tuberculosis*, *Klebsiella pneumoniae, Clostridium difficile,* or *Streptococcus pneumoniae*.

9. The isolated or synthesized protein fragment or peptide of claim 4 consisting essentially of at least one Replikin peptide or at least one homologue of said at least one Replikin peptide sequence.

10. The isolated or synthesized protein fragment or peptide of claim 4 consisting of one Replikin peptide sequence or one homologue of said Replikin peptide sequence.

11. The isolated or synthesized protein fragment or peptide of claim 4 comprising at least one of SEQ ID NO(s): 1-21, 22-56, or 57-119 or at least one homologue of SEQ ID NO(s): 1-21, 22-56, or 57-119.

12. The isolated or synthesized protein fragment or peptide of claim 4 consisting essentially of at least one Replikin peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119 or at least one homologue of SEQ ID NO(s): 1-21, 22-56, or 57-119.

13. The isolated or synthesized protein fragment or peptide of claim 5 comprising at least one functional fragment of at least one Replikin peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119.

14. The isolated or synthesized protein fragment or peptide of claim 4 consisting of 7 to 50 amino acid residues.

15. The isolated or synthesized protein fragment or peptide of claim 14 comprising at least one of SEQ ID NO(s): 1-21, 22-56, or 57-119.

16. The isolated or synthesized protein fragment or peptide of claim 4 consisting of 60, 70, 80, 90, 100, 150, or up to 200 amino acid residues.

17. The isolated or synthesized protein fragment or peptide of claim 4 that is chemically synthesized by solid phase methods.

18. An immunogenic and/or blocking composition comprising at least one protein, protein fragment, polypeptide, or peptide comprising at least one Replikin peptide sequence identified in a drug-resistant pathogen or at least one homologue of said at least one Replikin peptide sequence identified in a drug-resistant pathogen or at least one functional fragment of at least one Replikin peptide sequence identified in a drug-resistant pathogen.

19. The immunogenic and/or blocking compound of claim 18 comprising at least one peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119, at least one peptide sequence that is at least 80% homologous with at least one of SEQ ID NO(s): 1-21, 22-56, or 57-119, or at least one functional fragment of at least one of SEQ ID NO(s): 1-21, 22-56, or 57-119.

20. The immunogenic and/or blocking compound of claim 18 comprising at least one peptide consisting essentially of any one of SEQ ID NO(s): 1-21, 22-56, or 57-119.

21. A vaccine comprising at least one peptide sequence of any one of SEQ ID NO(s): 1-21, 22-56, or 57-119.

22. The vaccine of claim 21, comprising at least one peptide sequence consisting of at least one sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119.

23. The vaccine of claim 21 comprising a mixture of a plurality of peptide sequences of any of SEQ ID NO(s): 1-21, a mixture of a plurality of sequence of any of SEQ ID NO(s): 22-56, or mixture of plurality of peptide sequences of any of SEQ ID NO(s): 57-119, and/or a mixture of a plurality of homologues of peptide sequences of any of SEQ ID NO(s): 1-21, 22-56, or 57-119.

24. The vaccine of claim 21 comprising a mixture of a plurality of peptide sequences consisting of each of SEQ ID NO(s): 1-21, or each of SEQ ID NO(s): 22-56, or each of SEQ ID NO(s): 57-119.

25. The vaccine of claim 24 comprising an approximately equal molar mixture of the isolated or synthesized peptides of SEQ ID NO(s): 1-21, 22-56, or 57-119.

26. A binding agent that binds to at least a portion of an amino acid sequence of that is 50%, 60%, 70%, 80%, 90%, or 95% or more homologous with at least one Replikin peptide sequence identified in a drug-resistant pathogen.

27. The binding agent of claim 26, wherein said at least one Replikin peptide sequence is at least one peptide sequence of SEQ ID NO(s): 1-21, 22-56, or 57-119.

28. A method of making a vaccine comprising: selecting at least one isolated or synthesized protein, protein fragment, polypeptide, or peptide comprising at least one peptide sequence that is at least 50%, 60%, 70%, 80%, 90% or 95%, or 100% homologous with at least one Replikin peptide sequence identified in drug-resistant pathogen as a component of a vaccine; and making said vaccine.

29. The method of making a vaccine of claim 28 comprising selecting at least one isolated or synthesized peptide of SEQ ID NO(s): 1-21, 22-56, or 57-119, as at least one component of said vaccine and making said vaccine with said at least one component.

30. The method of making a vaccine of claim 28 comprising selecting at least two, three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, fifteen, sixteen, seventeen, eighteen, nineteen, twenty, or twenty-one or more isolated or synthesized Replikin peptide sequences identified in a drug-resistant pathogen and/or isolated or synthesized functional fragments of Replikin peptide sequences identified in a drug-resistant pathogen and making said vaccine comprising said selected sequence or functional fragments.

31. A method for preventing or treating drug-resistant pathogenic infection comprising administering at least one isolated or synthesized protein, protein fragment, polypeptide, or peptide comprising at least one peptide sequence, where the peptide sequence is at least 50%,

60%, 70%, 80%, 90% or 95%, or 100%, homologous with at least one Replikin peptide identified in a drug-resistant pathogen or a drug-resistant bacterial pathogen.

32. Use of at least one peptide sequence that is at least 50%, 60%, 70%, 80%, 90% or 95%, or 100%, homologous with at least one Replikin peptide identified in a drug-resistant pathogen in the manufacture of a medicament for preventing or treating drug-resistant pathogenic infection.

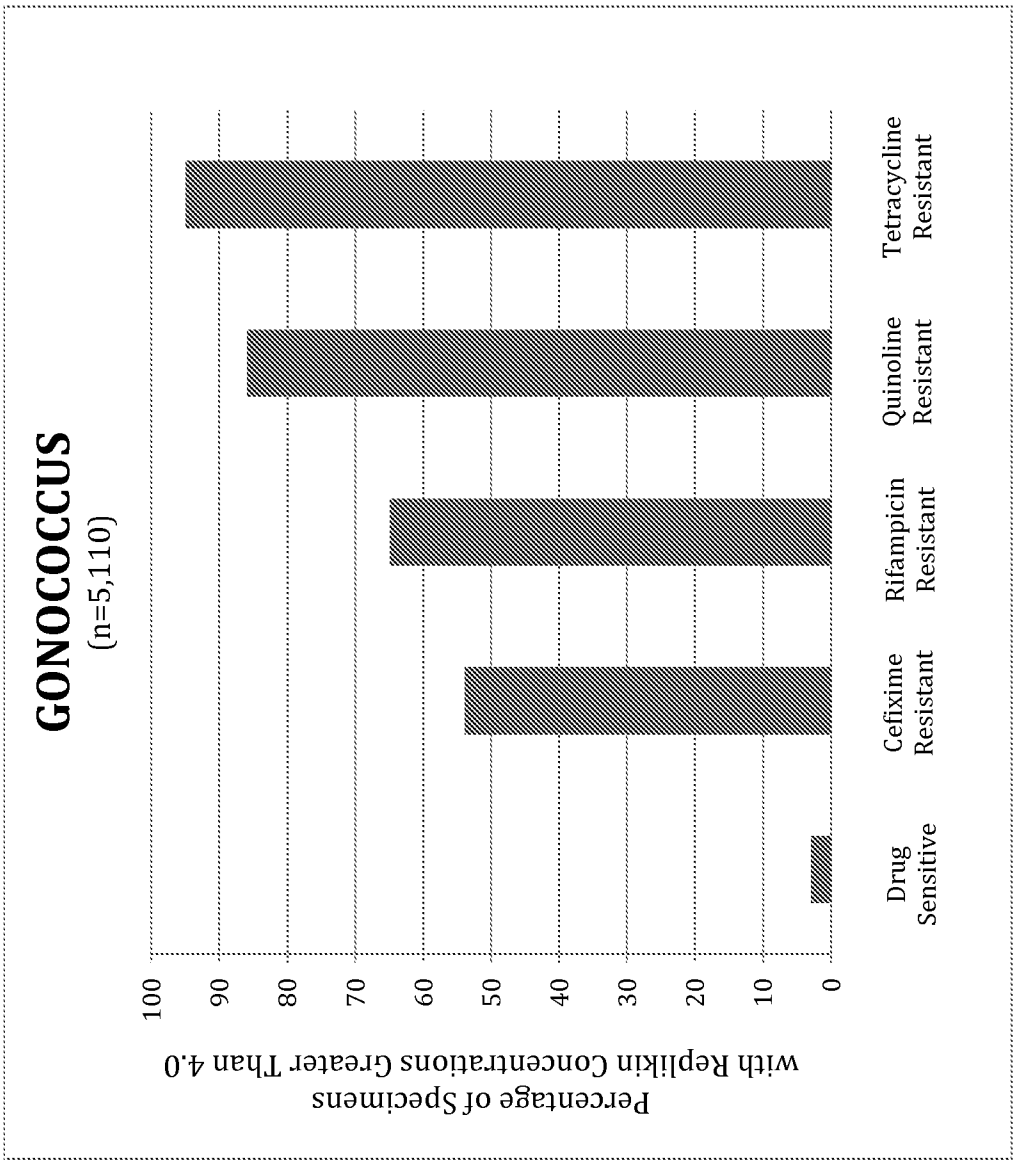
GONOCOCCUS
(n=5,110)
Percentage of Specimens with Replikin Concentrations Greater Than 4.0
100
90
80
70
60
50
40
30
20
10
0
Drug Sensitive
Cefixime Resistant
Rifampicin Resistant
Quinoline Resistant
Tetracycline Resistant

FIGURE 1

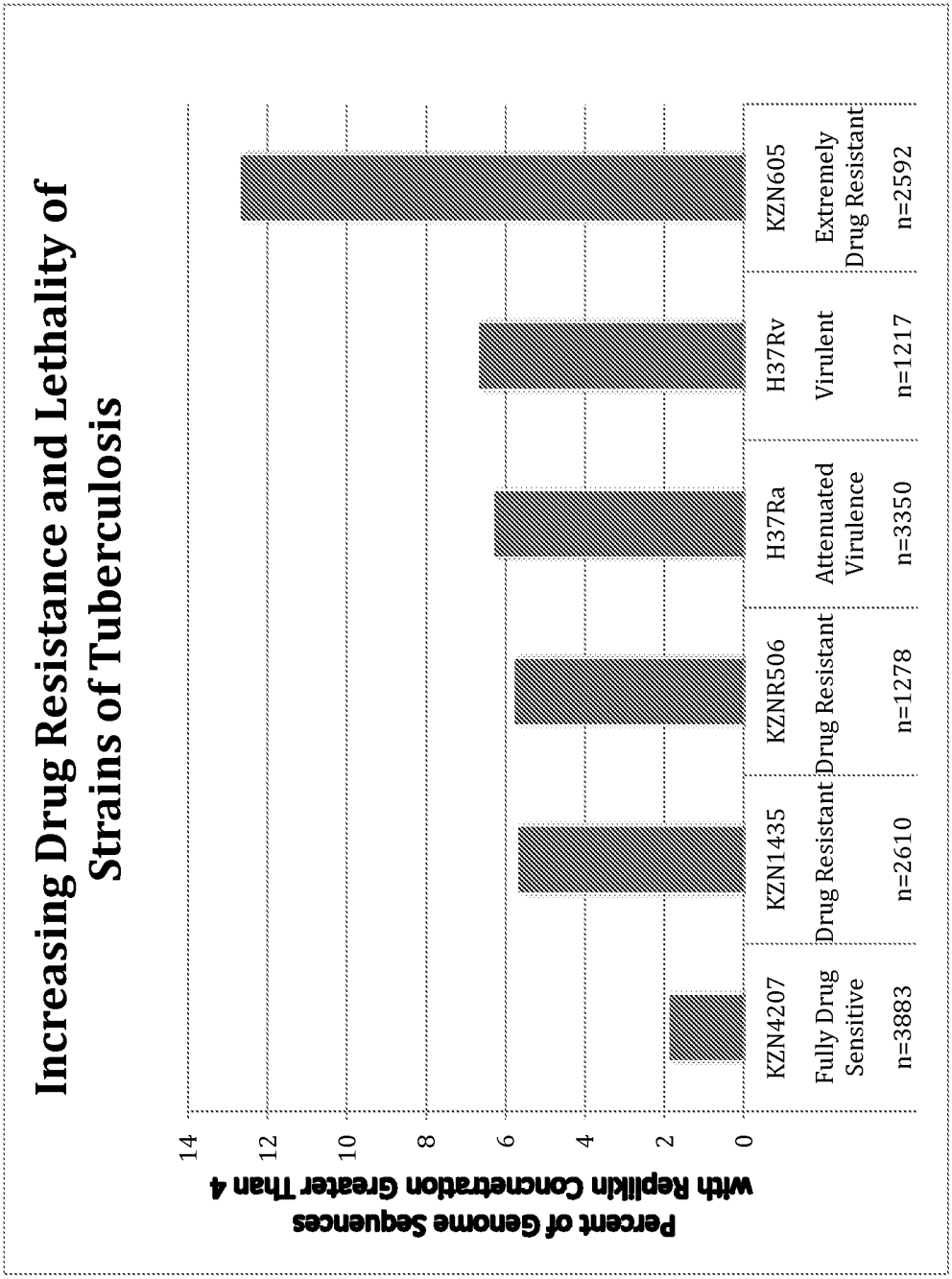
Increasing Drug Resistance and Lethality of Strains of Tuberculosis
Percent of Genome Sequences with Replikin Concentration Greater Than 4
0
2
4
6
8
10
12
14
KZN4207
Fully Drug Sensitive
n=3883
KZN1435
Drug Resistant
n=2610
KZNR506
Drug Resistant
n=1278
H37Ra
Attenuated Virulence
n=3350
H37Rv
Virulent
n=1217
KZN605
Extremely Drug Resistant
n=2592

FIGURE 2

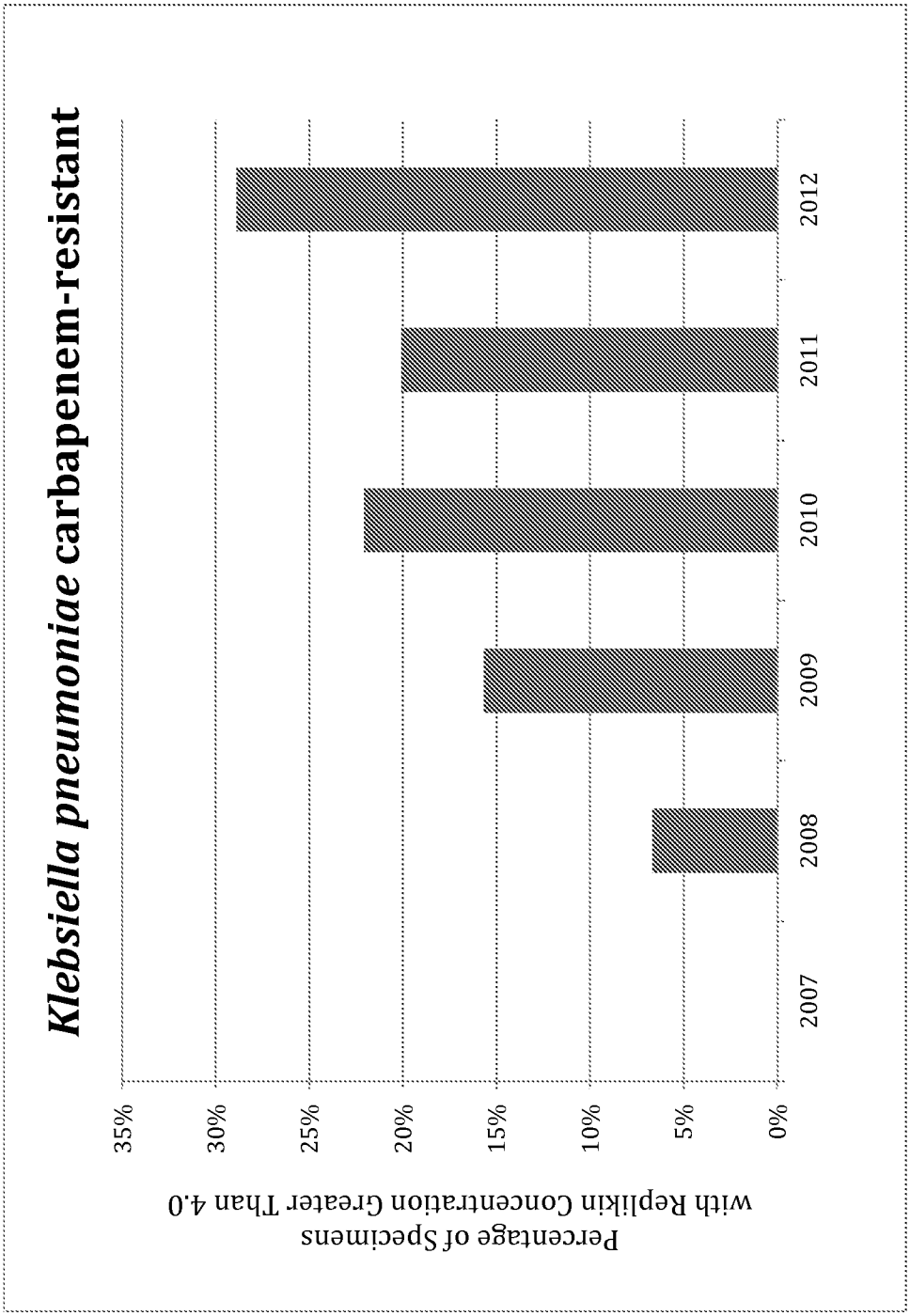
Klebsiella pneumoniae carbapenem-resistant
Percentage of Specimens with Replikin Concentration Greater Than 4.0
35%
30%
25%
20%
15%
10%
5%
0%
2007
2008
2009
2010
2011
2012

FIGURE 3

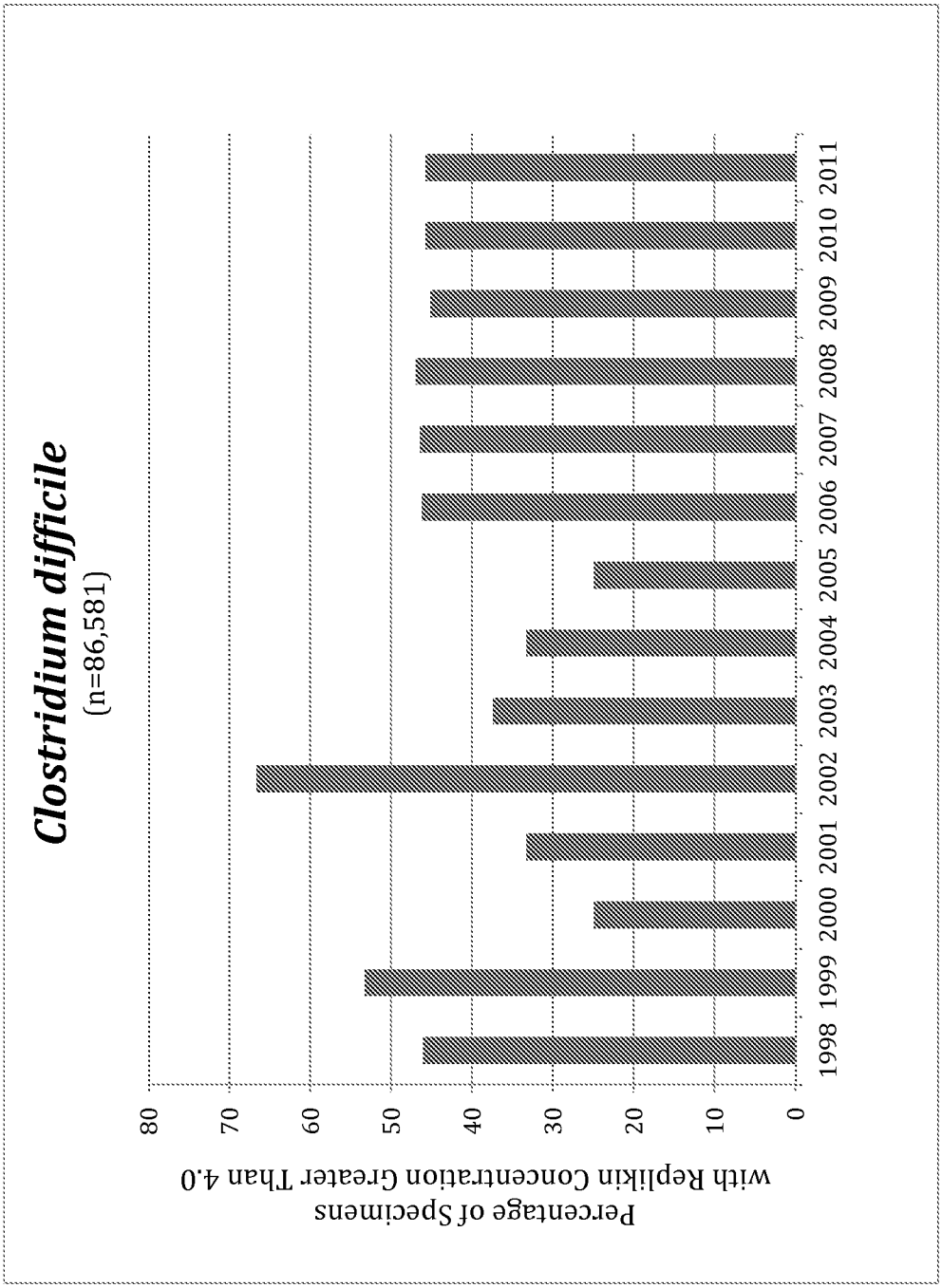
Clostridium difficile
(n=86,581)
Percentage of Specimens with Replikin Concentration Greater Than 4.0
0
10
20
30
40
50
60
70
80
1998
1999
2000
2001
2002
2003
2004
2005
2006
2007
2008
2009
2010
2011

FIGURE 4