ANTIBACTERIAL PHAGE, PHAGE PEPTIDES AND METHODS OF USE THEREOF

1. SEQUENCE LISTING

[0001] The instant application contains a Sequence Listing which has been submitted in ASCII format via EFS-Web and is hereby incorporated by reference in its entirety. Said ASCII copy, created on September 14, 2011, is named 16395US1.txt and is 3,295,858 bytes in size.

2. RELATED APPLICATION

[0002] This application claims priority from U.S. Provisional Application No. 61/384,015, filed on September 17, 2010, the contents of which are hereby incorporated by reference in their entirety.

3. FIELD OF THE INVENTION

[0003] The present invention is directed to the field of phage therapy for the treatment and control of bacterial infections. In particular, the present invention is directed to the novel bacteriophage F387/08, F391/08, F394/08, F488/08, F510/08, F44/10, F125/10, isolated polypeptides thereof, compositions comprising one or more of the novel bacteriophage and/or isolated polypeptides; and methods for the treatment and prevention of bacterial infections caused by, *e.g.*, *Staphylococcus aureus*, *Klebsiella pneumoniae, Acinetobacter baumannii*, *Escherichia coli*, and/or *Pseudomonas aeruginosa*, either alone or in combination with other therapies, *e.g.*, antibiotics or other phage therapies.

4. BACKGROUND

[0004] Bacteriophage (phage) are viruses that specifically infect and lyse bacteria. Phage therapy, a method of using whole phage viruses for the treatment of bacterial infectious diseases, was introduced in the 1920s by Felix d'Herelle. Initially, phage therapy was vigorously investigated and numerous studies were undertaken to assess the potential of phage therapy for the treatment of bacterial infection in humans and animals. Early success prompted the development of multiple commercial phage preparations. For example, in 1940 Eli Lilly Company produced 7 phage products for human use, including phage preparations for treating different sicknesses caused by *Staphylococcus* sp., *E. coli* and other pathogenic bacteria. These

preparations were used, for example, to treat infections that cause abscesses, purulent wounds, vaginitis, acute chronic upper-respiratory tract infections, and mastoid infections.

[0005] With the development of antibiotics in the 1940s, however, interest in phage-based therapeutics declined in the Western world. One of the most important factors that contributed to this decline was the lack of standardized testing protocols and methods of production. The failure to develop industry wide standards for the testing of phage therapies interfered with the documentation of study results, leading to a perceived lack of efficacy as well as problems of credibility regarding the value of phage therapy. Further, problems related to the production of phage samples/specimens complicated initial study and research. Diverse stabilizers and preservatives were initially used in attempts to increase the viability of the phage therapeutics. However, because the biology of both the phage and the various stabilizers were poorly understood, many of the ingredients added in an attempt to prolong the viability of phage preparations proved to be either toxic to humans or to negatively impact long term storage. Another problem in phage production related to the purity grade of the commercial preparations of the phage. At the time, phage therapy preparations generally consisted of raw lysates of host bacteria that had been treated with the phage of interest. Thus, many preparations contained what are now recognized to be undesired bacterial components, *e.g.*, endotoxins. Accordingly, adverse events were often associated with the preparations, particularly in patients receiving them intravenously. Nevertheless, in Eastern Europe and the former Soviet Union, where access to antibiotics was limited, the development and use of phage therapy continued jointly with, or in place of, antibiotics.

[0006] With the rise of antibiotic resistant strains of many bacteria, however, interest in phage-based therapeutics has returned in the Western world. Even though novel classes of antibiotics may be developed, the prospect that bacteria will eventually develop resistance to the new drugs has intensified the search for non-chemotherapeutic means for controlling, preventing, and treating bacterial infections. There are three main phage-based strategies for using phage therapy in a clinical environment: 1) administering virulent phage; 2) using endolysins or purified lysins encoded by bacteriophage 3) using structural proteins of phage as metabolic inhibitors of key bacterial enzymes, such as enzymes that synthesize peptidoglycan.

[0007] There is therefore a need to develop novel bacteriophage and phage products as potential therapeutic and/or prophylactic agents for use *in vivo* against pathogenic bacteria. In particular,

there is a need for bacteriophage capable of lysing nosocomial bacteria, including *Styphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Escherichia coli*, and/or *Pseudomonas aeruginosa*. Because most phage and phage peptides studied to date exhibit activity directed specifically against the species (or subspecies) of bacteria from which they are isolated, the novel phage-based therapies may find particular use in the hospital setting, selectively targeting nosocomial pathogens without affecting the normal surrounding flora.

5. SUMMARY

[0008] The present disclosure is directed to isolated bacteriophage and to isolated antibacterial polypeptides of bacteriophage origin for the treatment, prevention, or management of conditions associated with infection by Gram-positive or Gram-negative bacteria. In particular, the isolated bacteriophage or polypeptides of the disclosure may be used in pharmaceutical compositions for the treatment, prophylaxis, or management of infection by nosocomial pathogens, *e.g.*, Gram-negative bacteria including but not limited to *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Escherichia coli*, and *Pseudomonas aeruginosa*; and Gram-positive bacteria including but not limited to *Staphylococcus aureus*. In certain embodiments, the pharmaceutical compositions of the disclosure are of use in the treatment of conditions associated with infection by antibiotic resistant strains of bacteria, *e.g.*, methicillin resistant strains of *Staphylococcus aureus* (MRSA). In particular embodiments, the isolated bacteriophage or polypeptides of the disclosure are used for the topical treatment of infection by nosocomial pathogens in a subject in need thereof. In other embodiments, the isolated bacteriophage or polypeptides of the disclosure are used for the diagnosis of the infective agent in a sample (*e.g.*, tissue, blood, urine, sputum sample) derived from a patient. In other embodiments, the isolated bacteriophage or polypeptides of the disclosure are used as a prophylactic disinfectant or anti-infective for the preparation of solid surfaces, including skin or other epidermal surfaces.

[0008a] In one aspect, there is provided an isolated bacteriophage having a genome which comprises at least 99% sequence identity to the nucleotide sequence of SEQ ID NO:1, and having antibacterial activity against *Klebsiella pneumoniae*.

[0009] In certain embodiments, the disclosure provides an isolated bacteriophage, F391/08, having a genome comprising the nucleic acid sequence of SEQ ID NO:1 (FIGS. 15A-15III) and exhibiting antibacterial activity against one or more strains of *Klebsiella pneumoniae*. In other embodiments, the disclosure provides an isolated bacteriophage, F394/08, having a genome comprising the nucleic acid sequence of SEQ ID NO:2 (FIGS. 16A-16Q) and exhibiting antibacterial activity against one or more strains of *Acinetobacter baumannii*. In yet other embodiments, the disclosure provides an isolated bacteriophage, F488/08, having a genome

comprising the nucleic acid sequence of SEQ ID NO:3 (FIGS. 17A-17KKKK) and exhibiting antibacterial activity against one or more strains of *Escherichia coli*. In still yet other embodiments, the disclosure provides an isolated bacteriophage, F510/08, having a genome comprising the nucleic acid sequence of SEQ ID NO:4 (FIGS. 18A-18X) and exhibiting antibacterial activity against one or more strains of *Pseudomonas aeruginosa*. In still yet further embodiments, the disclosure provides an isolated bacteriophage, F44/10, having a genome comprising the nucleic acid sequence of SEQ ID NO:560 (FIGS. 19A-19UUU) and exhibiting antibacterial activity against one or more strains of *Staphylococcus aureus*. In still yet further embodiments, the disclosure provides an isolated bacteriophage, F387/08, having a genome comprising the nucleic acid sequence of SEQ ID NO:781 (FIGS. 20A-20KKKK) and exhibiting antibacterial activity against one or more strains of *Klebsiella pneumoniae*. In still yet further embodiments, the disclosure provides an isolated bacteriophage, F125/10, having a genome comprising the nucleic acid sequence of SEQ ID NO:1074 (FIGS. 21A-21ZZZ) and exhibiting antibacterial activity against one or more strains of *Staphylococcus aureus*.

[0010] The disclosure also encompasses isolated bacteria infected with one or more bacteriophage of the disclosure. In specific embodiments, the disclosure provides an isolated *K. pneumoniae* infected with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:1. In other embodiments, the disclosure provides an isolated *A. baumannii* infected with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:2. In still other embodiments, the disclosure provides an isolated *E. coli* infected with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:3. In yet other embodiments, the disclosure provides an isolated *P. aeruginosa* infected with one or more bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:4. In still yet other embodiments, the disclosure provides an isolated *S. aureus* infected with one or more bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO:560. In still yet further embodiments, the disclosure provides an isolated *K. pneumoniae* infected with one or more bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 781. In still yet further embodiments, the disclosure provides an isolated *S. aureus* infected with one or more bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 1074.

[0011] The present disclosure encompasses polypeptides isolated from bacteriophage F387/08, F391/08, F394/08, F488/08, F510/08, F44/10, and/or F125/10, which polypeptides exhibit antibacterial activity against one or more species or strains of Gram-positive or Gram-negative bacterium, *e.g.*, *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*. In specific embodiments, the polypeptides of the disclosure isolated or derived from F387/08 and F3910/08 exhibit antibacterial or antimicrobial activity, *e.g.*, lytic killing activity, against at least *K. pneumoniae*; those isolated or derived from F394/08, against at least *A. baumannii*; those isolated or derived from F488/08, against at least *E. coli*; those isolated or derived from F510/08 against at least *P. aeruginosa*; and those isolated or derived from F44/10 and F125/10 against at least *S. aureus*.

[0011a] In one aspect, there is provided an isolated protein from bacteriophage F391/08 having a genome comprising at least 99% sequence identity to the nucleotide sequence of SEQ ID NO:1, and having antibacterial activity against *Klebsiella pneumoniae*, or a fragment of said protein having antibacterial activity against *Klebsiella pneumoniae*; wherein said protein is a lysin protein or a tail protein.

[0011b] In one aspect, there is provided an isolated first protein having at least 85% or at least 95% sequence identity to a second protein of the same size, wherein said first protein has antibacterial activity against *Klebsiella pneumoniae,* and said second protein has the amino acid sequence of SEQ ID NO: 20, SEQ ID NO: 80, or a fragment thereof, or the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, or a fragment thereof.

[0011c] In one aspect, there is provided a fragment of the protein of the disclosure, wherein said fragment comprises a cysteine histidine-dependent amidohydrolases/peptidase domain (CHAP domain) of the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 80.

[0012] In certain embodiments, a polypeptide of the disclosure comprises or consists of an isolated lysin, or fragment thereof (*e.g.*, a CHAP domain) that exhibits antibacterial activity against one or more species or strains of bacteria, *e.g.*, Gram-positive bacteria, such as *S. aureus*; and/or Gram-negative bacteria, such as *K. pneumoniae A. baumannii*, *E. coli*, and/or *P. aeruginosa*. In specific embodiments, the polypeptide of the disclosure is an isolated lysin protein, *e.g.*, an endolysin or tail lysin, comprising or consisting of the amino acid sequence SEQ ID NO: 20, SEQ ID NO: 80, SEQ ID NO: 192, SEQ ID NO: 282, SEQ ID NO: 547, SEQ ID NO: 556, SEQ ID NO: 557, SEQ ID NO: 598, SEQ ID NO: 1216, or SEQ ID NO: 1261. Predicted functions of said lysin proteins include, for example an Ig-like virion protein (SEQ ID NO: 20), cell wall hydrolase (SEQ ID NO: 80), N-acetylmuramoyl-L-alanine amidase (SEQ ID NO: 192), soluble lysozyme (SEQ ID NO: 282), T4-like lysozyme (SEQ ID NO: 547), endolysin (SEQ ID NO: 556), lambda Rz1-like protein (SEQ ID NO: 557), endolysin (SEQ ID NO: 598), endolysin (SEQ ID NO: 1216), and tail lysin (SEQ ID NO: 1261).

[0013] In other embodiments, a polypeptide of the disclosure comprises a fragment, variant or derivative of SEQ ID NO: 20, SEQ ID NO: 80, SEQ ID NO: 192, SEQ ID NO: 282, SEQ ID NO: 547, SEQ ID NO: 556, SEQ ID NO: 557, SEQ ID NO: 598, SEQ ID NO: 1216, or SEQ ID NO: 1261, wherein said fragment, variant or derivative has antibacterial activity or antimicrobial activity, *e.g.*, lytic killing activity, against one or more strains of *K. pneumoniae A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*. In specific examples in accordance with this embodiment, the variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 20 and/or SEQ ID NO: 80 exhibits antibacterial or antimicrobial activity (*e.g.*, lytic killing activity) against one or

more strains of *K. pneumoniae*, for example, against the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 1. In other examples in accordance with this embodiment, the variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 192 exhibits antibacterial or antimicrobial activity (*e.g.*, lytic killing activity) against one or more strains of *A. baumannii*, for example, against the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 2. In other examples in accordance with this embodiment, the variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 282 exhibits antibacterial or antimicrobial activity (*e.g.*, lytic killing activity) against one or more strains of *E. coli*, for example, against the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 3. In other examples in accordance with this embodiment, the variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 547, SEQ ID NO: 556, and/or SEQ ID NO: 557 exhibits antibacterial or antimicrobial activity (*e.g.*, lytic killing activity) against one or more strains of *P. aeruginosa*, for example, against the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 4. In other examples in accordance with this embodiment, the variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 598, SEQ ID NO: 1216, and/or SEQ ID NO: 1261 exhibits antibacterial or antimicrobial activity (*e.g.*, lytic killing activity) against one or more strains of *S. aureus*, for example, against the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 560 or SEQ ID NO: 1074.

[0014] In specific embodiments, the isolated polypeptide of the disclosure comprises or consists of the CHAP domain of SEQ ID NO: 20, SEQ ID NO: 80, SEQ ID NO: 192, SEQ ID NO: 282, SEQ ID NO: 547, SEQ ID NO: 556, SEQ ID NO: 557, or SEQ ID NO: 598. In yet still other embodiments, a polypeptide of the disclosure comprises a fragment, variant or derivative of the CHAP domain of SEQ ID NO: 20, SEQ ID NO: 80, SEQ ID NO: 192, SEQ ID NO: 282, SEQ ID NO: 547, SEQ ID NO: 556, SEQ ID NO: 557, or SEQ ID NO: 598, wherein said fragment, variant or derivative has antibacterial activity or antimicrobial activity, *e.g.*, lytic killing activity, against at least one or more strains of *K. pneumoniae A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*.

[0015] In other embodiments, a polypeptide of the disclosure comprises or consists of an isolated tail protein (*e.g.*, tail component, tail fiber protein, tail length tape measure protein, adsorption

associated tail protein, major tail protein, major tail sheath protein, baseplate wedge subunit), or fragment thereof, having a biological function associated with the bacteriophage from which it is derived, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against at least one or more species or strains of *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*.

[0016] In specific embodiments, the polypeptide of the disclosure is an isolated tail protein comprising or consisting of the amino acid sequence SEQ ID NO: 15, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 629, SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, SEQ ID NOs: 1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1217, SEQ ID NO: 1250, or SEQ ID NO: 1266. In other embodiments, a polypeptide of the disclosure comprises a fragment, variant or derivative of SEQ ID NO: 15, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 629, SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, SEQ ID NOs: 1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1217, SEQ ID NO: 1250, or SEQ ID NO: 1266, wherein said fragment, variant or derivative exhibits a biological function associated with the bacteriophage from which it is derived, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*.

[0017] Predicted functions of said tail proteins include, for example, a receptor-binding tail protein (SEQ ID NO: 15), major tail protein (SEQ ID NO: 26 and SEQ ID NO: 1077), minor tail protein (SEQ ID NO: 27), pore-forming tail tip protein (SEQ ID NO: 30), tail protein (SEQ ID NOs: 32-33), minor tail protein (SEQ ID NO: 34), phage tail protein (SEQ ID NO: 35), tail sheath protein (SEQ ID NO: 180), tail tape measure protein (SEQ ID NO: 183), tail protein (SEQ

ID NO: 185), tail-fiber protein (SEQ ID NO: 190), tail tube protein (SEQ ID NO: 231), tail sheath monomer (SEQ ID NO: 232), tail sheath stabilizer and completion protein (SEQ ID NO:235), short tail fibers (SEQ ID NO: 239), base plate wedge completion tail pin (SEQ ID NOs: 240-241), base plate wedge completion tail fiber socket (SEQ ID NO: 242), base plate wedge subunit (SEQ ID NO: 243), base plate wedge initiator (SEQ ID NO: 244), base plate wedge (SEQ ID NO: 245), base plate hub subunit and tail lysozyme, cell-puncturing device (SEQ ID NO: 248), base plate wedge completion (SEQ ID NO: 249), tail completion and sheath stabilizer protein (SEQ ID NO: 252), chaperone long and short tail fiber assembly (SEQ ID NO: 254), tail fiber protein (SEQ ID NO: 433), tail fiber protein (SEQ ID NO: 434), hinge connecter long tail fiber (SEQ ID NO: 435), tail fiber hinge (SEQ ID NO: 436), proximal tail fiber subunit (SEQ ID NO: 437), base plate-tail tube initiatior (SEQ ID NO: 489), base plate (SEQ ID NO: 490), baseplate hub subunit, tail length determinator (SEQ ID NO: 491), base plate distal hub subunit (SEQ ID NO: 492), base plate hub subunit (SEQ ID NO: 493), base plate hub assembly catalyst (SEQ ID NO: 494), baseplate hub subunit (SEQ ID NO: 495), baseplate wedge subunit (SEQ ID NO: 496), tail tubular protein (SEQ ID NOs: 544-545), tail fiber protein (SEQ ID NO: 549 and SEQ ID NO: 551), major tail sheath protein (SEQ ID NO: 629 and SEQ ID NO: 1250), major tail protein (SEQ ID NO: 686), tail tube protein (SEQ ID NO: 789), fibritin (SEQ ID NO: 796), short tail fibers (SEQ ID NO: 797), base plate wedge completion tail pin (SEQ ID NO: 798), base plate wedge subunit and tail pin (SEQ ID NO: 799), baseplate wedge tail fiber connector (SEQ ID NO: 800), baseplate hub subunit and lysozyme (SEQ ID NO: 806), lysozyme (SEQ ID NO: 854), holin (SEQ ID NO: 999 and SEQ ID NO: 1217), distal long tail fiber assembly catalyst (SEQ ID NO: 1000), L-shaped tail fiber protein (SEQ ID NO: 1001), hinge connector of long tail fiber distal connector (SEQ ID NO: 1002), hinge connector of long tail fiber proximal connector (SEQ ID NO: 1003), long tail fiber proximal subnit (SEQ ID NO: 1004), baseplate tail tube initiator (SEQ ID NO: 1053), baseplate tail tube cap (SEQ ID NO: 1054), baseplate hub subunit, tail length determinator (SEQ ID NO: 1055), baseplate distal hub subunit (SEQ ID NO: 1056), baseplate hub subunit (SEQ ID NOs: 1057 and 1059), baseplate hub assemply catalyst (SEQ ID NO: 1058), baseplate wedge subunit (SEQ ID NO: 1060), and baseplate protein (SEQ ID NO: 1266).

[0018] In certain embodiments, the disclosure encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30,

or SEQ ID NOs: 32-35, which exhibits a biological function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 1, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *K. pneumoniae*. In other embodiments, the disclosure encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, or SEQ ID NO: 190, which exhibits a biological function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 2, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *A. baumannii*.

[0019] In certain embodiments, the disclosure encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NOs: 433-437, SEQ ID NOs: 489-495, or SEQ ID NO: 496, which exhibits a biological function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 3, *e.g.*, antimicrobial or antibacterial activity *(e.g.*, lytic killing activity), which function is directed against one or more strains of *E. coli*. In certain embodiments, the disclosure encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 549, or SEQ ID NO: 551, which exhibits a biological function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 4, *e.g.*, antimicrobial or antibacterial activity *(e.g.*, lytic killing activity), which function is directed against one or more strains of *P. aeruginosa*. In still other embodiments, the disclosure encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 629 or SEQ ID NO: 686, which exhibits a biological function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 560, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *S. aureus*. In still other embodiments, the disclosure encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, or SEQ ID NOs: 1053-1060, which exhibits a biological function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 781, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which

function is directed against one or more strains of *K. pneumoniae*. In still other embodiments, the disclosure encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 1077, SEQ ID NO: 1217, SEQ ID NO: 1250, or SEQ ID NO: 1266, which exhibits a biological function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 1074, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *S. aureus*.

[0020] In certain embodiments, the disclosure provides for isolated polypeptides that exhibit antimicrobial or antibacterial activity (*e.g.*, lytic killing activity) against one or more strains of bacteria, *e.g.*, Gram-positive bacteria (*e.g.*, *S. aureus*), Gram-negative bacteria (*e.g.*, *K. pneumoniae*, *A. baumannii*, *E. coli*, and *P. aeruginosa*) or bacteria not classified as either Gram-positive or Gram-negative, wherein the isolated polypeptides have an amino acid sequence with at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater sequence identity to a second amino acid sequence of the same length (*i.e.*, consisting of the same number of residues), which second amino acid sequence is of SEQ ID NO: 15, SEQ ID NO: 20, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, SEQ ID NO: 80, SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 282, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 547, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 556, SEQ ID NO: 557, SEQ ID NO: 598, SEQ ID NO: 629, SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, SEQ ID NOs: 1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1216, SEQ ID NO: 1217, SEQ ID NO: 1250, SEQ ID NO: 1261, SEQ ID NO: 1266, and/or a fragment thereof.

[0021] The disclosure further provides isolated polypeptides comprising or consisting of the amino acid sequence of any of SEQ ID NOs: 5-176, SEQ ID NOs: 177-223, SEQ ID NOs: 224-506, SEQ ID NOs: 507-559, SEQ ID NOs: 561-780, SEQ ID NOs: 782-1073, and SEQ ID NOs: 1075-1300. In other embodiments, isolated polypeptides of the disclosure recombinantly fused or chemically conjugated (*e.g.*, covalent or non-covalent conjugation) to therapeutic agents (*e.g.*, heterologous polypeptides or small molecules) are provided.

[0022] The disclosure also encompasses polynucleotides that encode the polypeptides of the disclosure. In one aspect, there is provided an isolated nucleic acid comprising a nucleotide sequence encoding the protein or fragment of the disclosure, In a specific embodiment, the disclosure provides an isolated nucleic acid comprising a nucleic acid sequence encoding the polypeptide of any of SEQ ID NOs: 5-176, SEQ ID NOs: 177-223, SEQ ID NOs: 224-506, SEQ ID NOs: 507-559, SEQ ID NOs: 561-780, SEQ ID NOs: 782-1073, and SEQ ID NOs: 1075-1300. In other embodiments, the disclosure provides an isolated nucleic acid comprising a nucleic acid sequence encoding the polypeptide of any of SEQ ID NO: 15, SEQ ID NO: 20, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, SEQ ID NO: 80, SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 282, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 547, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 556, SEQ ID NO: 557, SEQ ID NO: 598, SEQ ID NO: 629, SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, SEQ ID NOs: 1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1216, SEQ ID NO: 1217, SEQ ID NO: 1250, SEQ ID NO: 1261, or SEQ ID NO: 1266, or active fragment, variant or derivative thereof, which polypeptide or active fragment, variant or derivative exhibits a biological function associated with the bacteriophage from which it is isolated and/or derived, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity). The disclosure also relates to a vector comprising one or more of said nucleic acids. In one specific embodiment, said vector is an expression vector. The disclosure further provides host cells containing a vector comprising one or more polynucleotides one or more encoding the polypeptides of the disclosure.

[0023] The present disclosure encompasses methods for the production of polypeptides of the disclosure or active fragments thereof, in particular for use in pharmaceutical compositions, *i.e.*, antimicrobial compositions. For example, the polypeptides of the disclosure may be isolated directly from cell cultures (*e.g.*, bacterial cell cultures) infected with bacteriophage F387/08, F391/08, F394/08, F488/08, F510/08, F44/10, and/or F125/10. Alternatively, the polypeptides of the present disclosure may be derived by recombinant means using expression vectors comprising nucleic acid sequence encoding polypeptides of the disclosure, *e.g.*, SEQ ID NO: 15, SEQ ID NO: 20, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, SEQ ID NO: 80, SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 192,

SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 282, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 547, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 556, SEQ ID NO: 557, , SEQ ID NO: 598, SEQ ID NO: 629, SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, SEQ ID NOs: 1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1216, SEQ ID NO: 1217, SEQ ID NO: 1250, SEQ ID NO: 1261, or SEQ ID NO: 1266, or active fragments, derivatives or variants thereof. The polypeptides of the disclosure or fragments thereof can be produced by any method known in the art for the production of a polypeptide, in particular, by chemical synthesis or by recombinant expression techniques.

[0024] In specific embodiments, the disclosure relates to a method for recombinantly producing a phage protein, *e.g.*, a lysin protein, tail protein, or active fragment, variant or derivative thereof, said method comprising: (i) culturing under conditions suitable for the expression of said protein in a medium, a host cell containing a vector comprising a nucleic acid sequence encoding the amino acid sequence SEQ ID NO: 15, SEQ ID NO: 20, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, SEQ ID NO: 80, SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 282, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 547, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 556, SEQ ID NO: 557, SEQ ID NO: 598, SEQ ID NO: 629, SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, or SEQ ID NOs: 1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1216, SEQ ID NO: 1217, SEQ ID NO: 1250, SEQ ID NO: 1261, or SEQ ID NO: 1266, or fragment thereof; and (ii) recovery of said protein from said medium. In certain embodiments, the nucleic acid sequence encoding the polypeptide of the disclosure is operably linked to a heterologous promoter.

[0025] The disclosure also encompasses methods for the diagnosis of the causative agent in a clinical presentation of bacterial infection. The isolated bacteriophage or polypeptides of the disclosure may be used to aid in the determination of species of bacteria in a patient sample by establishing susceptibility of the bacteria in the sample to the bacteriophage and/or polypeptides of the disclosure. Such methods further encompass methods of evaluation of antibacterial activity

of the isolated bacteriophage and/or polypeptides of the disclosure. Antibacterial activity of the bacteriophage or the polypeptides of the disclosure, or susceptibility of an unknown sample to such activity, may be assessed by any method known in the art and/or described herein. In certain embodiments, antibacterial activity and/or susceptibility is assessed by culturing known bacteria and/or patient tissue, blood, fluid or swab samples according to standard techniques (*e.g.*, in liquid culture or on agar plates), contacting the culture with bacteriophage and/or polypeptides of the disclosure and monitoring cell growth after said contacting. For example, in a liquid culture, the bacteria (*e.g.*, *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*) may be grown to a optical density ("OD") representative of a mid-point in exponential growth of the culture; the culture is exposed to one or more concentrations of one or more bacteriophage and/or polypeptides of the disclosure and the OD is monitored relative to a control culture. Decreased OD relative to a control culture is representative of a bacteriophage and/or polypeptide exhibiting antibacterial activity (*e.g.*, exhibiting lytic killing activity) against the tested sample or bacterial species and/or strain in the culture. Similarly, bacterial colonies can be allowed to form on an agar plate, the plate exposed to a bacteriophage or polypeptide of the disclosure, and subsequent growth of the colonies evaluated relative to control plates. Decreased size of colonies, or decreased total numbers of colonies, indicates a bacteriophage and/or polypeptide with antibacterial activity against the tested sample and/or cultured species or strain.

[0025a] In one aspect, there is provided a method for diagnosing the causative agent of a bacterial infection comprising

(i) culturing a sample from a patient;

(ii) contacting the culture of step (i) with the bacteriophage of the disclosure, the protein or fragment of the disclosure; and

(iii) monitoring for evidence of growth or lysis of the culture

wherein evidence of lysis of the culture indicates that the culture comprises *Klebsiella pneumoniae*;

preferably wherein the sample is a sample of blood, urine, tissue biopsy, sputum, nasal mucosa, or swab collected from said patient.

[0025b] In one aspect, there is provided a pharmaceutical composition comprising the bacteriophage of the disclosure and a pharmaceutically acceptable carrier.

[0026] The present disclosure is also directed to pharmaceutical compositions comprising or consisting of a bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 560, SEQ ID NO: 781, or SEQ ID NO: 1074. In certain embodiments, the pharmaceutical composition of the disclosure comprises a bacteriophage having the genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 560, SEQ ID NO: 781, or SEQ ID NO: 1074, in addition to one or more other bacteriophage. The one or more other bacteriophage may be one or more bacteriophage of the disclosure (*e.g.*, having a genome comprising or consisting of a nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 560, SEQ ID NO: 781, or SEQ ID NO: 1074), one or more strains thereof, or may be one or more bacteriophage known in the art other than a bacteriophage having a genome according to SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 560, SEQ ID NO: 781, or SEQ ID NO: 1074.

Further, the one or more bacteriophage in the pharmaceutical composition of the disclosure may target the same or different species or strains of bacteria. In certain embodiments, the pharmaceutical compositions comprising one or more bacteriophage of the disclosure further comprise one or more polypeptides of the disclosure and/or other phage products as described herein or known in the art.

[0026a] In one aspect, there is provided a pharmaceutical composition comprising the isolated protein or fragment of the disclosure and a pharmaceutically acceptable carrier.

[0027] In certain embodiments, the disclosure provides pharmaceutical compositions comprising polypeptides, or active fragments thereof, in particular those having anti-microbial and/or antibacterial activity, isolated from bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 560, SEQ ID NO: 781, and/or SEQ ID NO: 1074. In specific embodiments, the pharmaceutical compositions of the disclosure comprise one or more polypeptides having an amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 20, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, SEQ ID NO: 80, SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 282, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 547, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 556, SEQ ID NO: 557, SEQ ID NO: 598, SEQ ID NO: 629, SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, SEQ ID NOs: 1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1216, SEQ ID NO: 1217, SEQ ID NO: 1250, SEQ ID NO: 1261, or SEQ ID NO: 1266. In other embodiments, the pharmaceutical compositions of the disclosure comprise a polypeptide that is a variant, derivative or fragment of SEQ ID NO: 15, SEQ ID NO: 20, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, SEQ ID NO: 80, SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 282, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 547, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 556, SEQ ID NO: 557, SEQ ID NO: 598, SEQ ID NO: 629, SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, SEQ ID NOs: 1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1216, SEQ ID NO: 1217, SEQ ID NO: 1250, SEQ ID NO: 1261, or SEQ ID NO: 1266,

wherein the variant, derivative or fragment retains a biological function of the polypeptide from which it is derived, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), preferably against one or more strains of *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*.

[0028] The pharmaceutical compositions of the disclosure may additionally comprise a pharmaceutically acceptable carrier, excipient, or stabilizer. In certain embodiments, the pharmaceutical compositions of the disclosure are antibiotic compositions (in that they exhibit antibacterial activity) or therapeutic compositions for the treatment, prevention, and/or amelioration of symptoms of a disease or disorder associated with infection by bacteria in a subject in need thereof. In specific embodiments, the pharmaceutical compositions of the disclosure are antibacterial compositions or therapeutic compositions for the treatment, prevention, and/or amelioration of symptoms of a disease or disorder associated with infection by *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*. In certain embodiments, the subject receiving a pharmaceutical composition of the disclosure is a mammal (*e.g.*, bovine, ovine, caprine, equid, primate (*e.g.*, human), rodent, lagomorph or avian (*e.g.*, chicken, duck, goose)).

[0029] The present disclosure provides for methods for the treatment or prevention of bacterial infection comprising administering to a subject in need thereof a pharmaceutical composition comprising one or more bacteriophage or phage products (*e.g.*, an isolated bacteriophage polypeptide or active fragment, variant or derivative thereof), optionally in addition to one or more other bacteriophage or other phage products, as described herein. In the context of the present disclosure, "treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to eliminate, lessen, decrease the severity of, slow the progression of or delay or prevent the symptoms or underlying cause (*e.g.*, bacterial infection) associated with the pathological condition or disorder. The pharmaceutical compositions of the present disclosure may be used in the treatment or management of infections associated with any bacterial infection, including, but not limited to *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*, as well as, in certain embodiments, *S. epidermidis*, *S. auricularis*, *S. capitis*, *S. haemolyticus*, *S. hominis*, *S. saprophyticus*, *S. simulans*, *S. xylosis*, *M. luteus*, *B. subtilis*, *B. pumilus*, *E. faecalis*, *E. hirae*, *E. faecium*, *E. avium*, and combinations thereof. In certain embodiments, the pharmaceutical compositions may be used to

treat conditions or disorders associated with bacterial infections including, but not limited to, post-operative endophtalmitis, endocarditis, infections of the central nervous system, pneumonia, osteomylelitis, wound infections (*e.g.*, diabetic foot ulcers), mastitis, septicemia, food poisoning, meningitis, skin infections, abscesses, toxic shock syndrome, bacteremia, and/or other conditions associated with nosocomial bacterial infections.

[0030] In certain embodiments, the disclosure provides for the use of a bacteriophage or an isolated phage product (*e.g.*, an isolated phage polypeptide or active fragment, variant or derivative thereof) as a single agent therapy. In other embodiments, the disclosure provides for the use of a bacteriophage, or phage product (*e.g.*, an isolated phage polypeptide or active fragment, variant or derivative thereof), in combination with a standard or experimental treatment for bacterial infection. Such combination therapy may enhance the efficacy of the standard or experimental treatment. Examples of therapeutic agents that are particularly useful in combination with a bacteriophage and/or polypeptide of the disclosure are anti-inflammatory agents, standard chemotherapeutic antibiotic agents (*e.g.*, penicillin, synthetic penicillins, bacitracin, methicillin, nafcillin, oxacilin, cloxacillin, vancomycin, teicoplanin, clindamycin, co-trimoxazole, cephalosporin, polymyxin, cefaclor. Cefadroxil, cefamandole nafate, cefazolin, cefixime, cefmetazole, cefonioid, cefoperazone, ceforanide, cefotanme, cefotaxime, cefotetan, cefoxitin, cefpodoxime proxetil, ceftazidime, ceftizoxime, ceftriaxone, cefriaxone moxalactam, cefuroxime, cephalexin, cephalosporin C, cephalosporin C sodium salt, cephalothin, cephalothin sodium salt, cephapirin, cephradine, cefuroximeaxetil, dihydratecephalothin, moxalactam, loracarbef mafate and chelating agents), local anesthetic agents, and/or corticosteroids. In yet another embodiment, the compositions of the present disclosure may be combined with one or more bacteriophage or phage products known in the art. The combination therapies encompassed by the disclosure may be formulated into a single pharmaceutical composition or may be administered in separate compositions, but as part of an overall treatment regimen.

[0031] The pharmaceutical compositions of the disclosure may be administered by any method known in the art suitable for administration of an antibacterial compound, *e.g.*, via oral or parenteral (*e.g.*, inhalation, intramuscular, intravenous, or epidermal) delivery. In preferred embodiments, the pharmaceutical compositions of the disclosure are administered topically, *e.g.*, in a topical formulation. The compositions of the disclosure may be used topically to treat and/or prevent common nosocomial infections, such as infections at surgical incision sites or associated

with catheters or drains. In other embodiments, the compositions of the disclosure are use to treat bacterial infections of the skin or upper dermal layers (*e.g.*, infections of diabetic ulcers of the foot or carbuncles).

[0031a] In one aspect, there is provided use of a therapeutically effective amount of the pharmaceutical composition of the disclosure in the manufacture of a medicament for treating or preventing a bacterial infection in a subject in need thereof; wherein said bacterial infection is an infection by *Klebsiella pneumonia,* preferably wherein the infection is a nosocomial infection and/or wherein the subject is a mammal, preferably a human.

[0031b] In one aspect, there is provided use of the bacteriophage of the disclosure, the polypeptide or fragment of the disclosure in the manufacture of a medicament for reducing or inhibiting colonization or growth of *Klebsiella pneumoniae* bacteria on a biological surface contacted therewith; preferably wherein said surface is the skin or a mucus membrane of a mammal, the skin or a mucus membrane of a human, or a mucus membrane of the respiratory tract of a mammal or human.

[0032] The pharmaceutical compositions of the present disclosure may also be used for traditionally non-therapeutic uses such as antibacterial agents in cosmetics, or in sprays or solutions for use on solid surfaces to prevent the colonization of bacteria (*i.e.*, as disinfectants).

[0032a] In one aspect, there is provided a method for reducing or inhibiting colonization or growth of *Klebsiella pneumoniae* bacteria on a solid surface comprising contacting said surface with the bacteriophage of the disclosure, the polypeptide or fragment of the disclosure; preferably wherein said surface is the surface of a hospital apparatus or a piece of hospital equipment, preferably a surgical apparatus or piece of surgical equipment.

[0033] The present disclosure is also directed to methods for screening peptides for antibacterial activity. In one embodiment the method comprises screening contiguous amino acid sequences of at least 6, 10, 15, 20 or 25 residues in length that are encoded by the open reading frames of the nucleic acid sequence SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 560, SEQ ID NO: 781, or SEQ ID NO: 1074, for antibacterial activity, said antibacterial activity measured by the peptides ability to inhibit bacterial growth, *e.g.*, in agar or liquid culture.

5.1 DEFINITIONS

[0034] As used herein, the term "fragment" refers to a peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least 80 contiguousamino acid residues, at least 90 contiguous amino acid residues, at least 100 contiguous amino acid residues, at least 125 contiguous amino acid residues, at least 150 contiguous amino acid residues, at least 175 contiguous amino acid residues, at least 200 contiguous amino acid residues, or at least 250 contiguous amino acid residues of the amino acid sequence of a protein. In a specific embodiment, the fragment is a functional fragment in that it retains at least one function of the protein from which it is isolated (*e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic cell killing)).

[0035] As used herein the terms "active bacteriophage products" and "bacteriophage products" refer to polypeptides, or fragments, variants or derivatives thereof, isolated from a bacteriophage of the invention, which polypeptide, or fragment, variant or derivative thereof, exhibits a biological function or activity associated with the bacteriophage from which it was isolated or derived (*e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic cell killing)).

[0036] As used herein, the term "isolated" in the context of a peptide, polypeptide, or fusion protein or refers to a peptide, polypeptide or fusion protein that is substantially free of cellular material or contaminating proteins from the cell or tissue source from which it is derived, or substantially free of chemical precursors or other chemicals when chemically synthesized. The language "substantially free of cellular material" includes preparations of a peptide, polypeptide or fusion protein in which the peptide, polypeptide or fusion protein is separated from cellular components of the cells from which it is isolated or recombinantly produced. Thus, a peptide, polypeptide or fusion protein that is substantially free of cellular material includes preparations of a peptide, polypeptide or fusion protein having less than about 30%, 20%, 10%, or 5% (by dry weight) of heterologous protein (also referred to herein as a "contaminating protein"). When the peptide, polypeptide or fusion protein is recombinantly produced, it is also preferably substantially free of culture medium, *i.e.*, culture medium represents less than about 20%, 10%, or 5% of the volume of the protein preparation. When the peptide, polypeptide or fusion protein is produced by chemical synthesis, it is preferably substantially free of chemical precursors or other chemicals, *i.e.*, it is separated from chemical precursors or other chemicals which are involved in the synthesis of the peptide, polypeptide, or fusion protein. Accordingly, such preparations of a peptide, polypeptide, fusion protein, or antibody have less than about 30%, 20%, 10%, 5% (by dry weight) of chemical precursors or compounds other than the peptide, polypeptide, or fusion protein of interest.

[0037] As used herein, the term "isolated" in the context of nucleic acid molecules refers to a first nucleic acid molecule which is separated from other nucleic acid molecules which are present in the natural source of the first nucleic acid molecule. Moreover, an "isolated" nucleic acid molecule, such as a cDNA molecule, is substantially free of other cellular material, or culture medium when produced by recombinant techniques, or substantially free of chemical precursors or other chemicals when chemically synthesized and may be free of other cDNA or other genomic DNA molecules, *e.g.*, where it has been isolated from other clones in a nucleic acid library.

[0038] The term "purified" means that the peptide, polypeptide, fusion protein, or nucleic acid molecule has been measurably increased in concentration by any purification process, including but not limited to, column chromatography, HPLC, precipitation, electrophoresis, etc., thereby partially, substantially, nearly completely, or completely removing impurities, such as precursors

or other chemicals involved in preparing the peptide, polypeptide, fusion protein, or nucleic acid molecule. One of skill in the art will appreciate the amount of purification necessary for a given use. For example, isolated protein meant for use in therapeutic compositions intended for administration to humans ordinarily must be of high purity in accordance with regulatory standards and good manufacturing processes.

[0039] As used herein, the term "derivative" in the context of polypeptides refers to a polypeptide that comprises an amino acid sequence which has been altered by the introduction of amino acid residue substitutions, deletions or additions. The term "derivative" as used herein also refers to a polypeptide that has been modified, *i.e.*, by the covalent attachment of any type of molecule to the polypeptide. For example, but not by way of limitation, a polypeptide may be modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. A derivative polypeptide may be produced by chemical modifications using techniques known to those of skill in the art, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Further, a derivative polypeptide may contain one or more non-classical amino acids. A polypeptide derivative possesses a similar or identical function as the polypeptide from which it was derived. The term "derived" as used in reference to a polypeptide "derived" from an organism may also refer to isolation of a polypeptide directly from said organism (*e.g.* bacterial cells or phage).

[0040] As used herein, the term "host cell" refers to the particular subject cell transfected with a nucleic acid molecule and the progeny or potential progeny of such a cell that contain the nucleic acid molecule or chromosomally integrated version thereof. Progeny of such a cell may not be identical to the parent cell transfected with the nucleic acid molecule due to mutations or environmental influences that may occur in succeeding generations or integration of the nucleic acid molecule into the host cell genome. For the expression of bacteriophage proteins and polypeptides, the host cell is preferably not of the same species or strain from which the bacteriophage was isolated or cultured.

[0041] As used herein, the term "in combination" refers to the use of more than one prophylactic and/or therapeutic agent. The use of the term "in combination" does not restrict the order in which prophylactic and/or therapeutic agents are administered to a subject with a disease or

disorder. A first prophylactic or therapeutic agent can be administered prior to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second prophylactic or therapeutic agent (different from the first prophylactic or therapeutic agent) to a subject in need thereod, *e.g.*, a subject with a disease or disorder.

[0042] As used herein, the terms "nucleic acids" and "nucleotide sequences" include single-stranded and double-stranded DNA and/or RNA molecules, or combinations thereof. As used herein, the term "encoded by the nucleic acid" refers to an amino acid sequence that results from the translation of the forward, reverse, complementary or reverse-complementary sequence of the referenced nucleic acid sequence using the standard genetic code (*i.*e., standard codon triplets) as well known in the art.

[0043] As used herein, the terms "prophylactic agent" and "prophylactic agents" refer to bacteriophage and/or polypeptides of the invention, which can be used in the prevention, treatment, management or amelioration of one or more symptoms of a disease or disorder, in particular, a disease or disorder associated with a bacterial infection.

[0044] As used herein, the terms "therapeutic agent" and "therapeutic agents" refer to bacteriophage and/or polypeptides of the invention that can be used in the prevention, treatment, management or amelioration of one or more symptoms of a disease or disorder, in particular, a disease or disorder associated with a bacterial infection.

[0045] As used herein, the term "therapeutically effective amount" refers to that amount of a therapeutic agent sufficient to result in amelioration of one or more symptoms of a disease or disorder, in particular, a disease or disorder associated with a bacterial infection.

[0046] As used herein, the terms "treat", "treatment" and "treating" refer to the amelioration of one or more symptoms of a disease or disorder, in particular, a disease or disorder associated with a bacterial infection, which results from the administration of one or more bacteriophage and/or polypeptides of the invention. As noted above, "treatment" and related terms refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to eliminate, lessen, decrease the severity of, slow the progression of, or delay or prevent the

symptoms or underlying cause (*e.g.*, bacterial infection) associated with the pathological condition or disorder.

[0047] As used herein, the terms "antibacterial activity" and "antimicrobial activity" with reference to a bacteriophage, isolated bacteriophage protein (or variant, derivative or fragment thereof), or bacteriophage product, are used interchangeably to refer to the ability to kill and/or inhibit the growth or reproduction of a microorganism, in particular, the bacteria of the species or strain that the bacteriophage infects. In certain embodiments, antibacterial or antimicrobial activity is assessed by culturing bacteria, *e.g.*, Gram-positive bacteria (*e.g.*, *S. aureus*), Gram-negative bacteria (*e.g.*, *K. pneumoniae, A. baumannii, E. coli*, and/or *P. aeruginosa*) or bacteria not classified as either Gram-positive or Gram-negative, according to standard techniques (*e.g.*, in liquid culture or on agar plates), contacting the culture with a bacteriophage or polypeptide of the invention and monitoring cell growth after said contacting. For example, in a liquid culture, the bacteria may be grown to an optical density ("OD") representative of a mid-point in exponential growth of the culture; the culture is exposed to one or more concentrations of one or more bacteriophage or polypeptides of the invention, and the OD is monitored relative to a control culture. Decreased OD relative to a control culture is representative of a bacteriophage or polypeptide exhibiting antibacterial activity (*e.g.*, exhibits lytic killing activity). Similarly, bacterial colonies can be allowed to form on an agar plate, the plate exposed to a bacteriophage or polypeptide of the invention, and subsequent growth of the colonies evaluated related to control plates. Decreased size of colonies, or decreased total numbers of colonies, indicate a bacteriophage or polypeptide with antibacterial activity.

[0048] As used herein, a "CHAP domain" refers to a conserved amidase domain found in several phage-encoded peptidoglycan hydrolases and stands for for "cysteine, histidine-dependent amidohydrolases/peptidases." See, *e.g.*, Rigden D, et. al., Trends Biochem Sci. 2003 May 28(5): 230-4. It is found in a superfamily of amidases, including GSP amidase and peptidoglycan hydrolases. The family includes at least two different types of peptidoglycan cleavage activities: L-muramoyl-L-alanine amidase and D-alanyl-glycyl endopeptidase activity. CHAP domains generally contain conserved cysteine and histidine residues and hydrolyze γ-glutamyl-containing substrates. These cysteine residues are believed to be essential for the activity of several of these amidases, and their thiol groups appear to function as the nucleophiles in the catalytic mechanisms of all enzymes containing this domain. CHAP domains are often found in

association with other domains that cleave peptidoglycan, *e.g.*, acting in a cooperative manner to cleave specialized substrates. See also, Bateman A, et al., Trends Biochem Sci. 2003 May 28(5): 234-7.

6. BRIEF DESCRIPTION OF THE FIGURES

[0049] FIGS. 1A-1B: Schematic of the organization of the F391/08 genome, comprising the nucleic acid sequence of SEQ ID NO:1. The open reading frames ("ORFs") predicted in the approximately 113kb genome are represented by arrows and numbered in black. The direction of an arrow indicates the direction of transcription. Color coding: Black – ORFs for which products a functional assignment could be made based on the known functions of homologous proteins; Gray - ORFs coding for products that are similar to proteins of unknown function; Empty – ORFs coding for proteins that share no significant homology with proteins in available databases. Functionally assigned ORFs are also listed in the figure. The information in the figure is also included in tabular form in FIG. 2.

[0050] FIGS. 2A-2II: Features of the bacteriophage F391/08 genome, including gene products and assignment of putative functions. The figure includes a listing of the ORFs of the genome and provides for each ORF (i) its position within the genome, (ii) the encoded amino acid sequence, (iii) a listing of homologous proteins and conserved domains within its encoded polypeptide and (iv) an assignment of putative function. ORFs 1-172 listed in FIG. 2 encode the amino acid sequences of SEQ ID NO:5-176, respectively.

[0051] FIG. 3: Schematic of the organization of the F394/08 genome, comprising the nucleic acid sequence of SEQ ID NO:2. The open reading frames ("ORFs") predicted in the approximately 31kb genome are represented by arrows and numbered in black. The direction of an arrow indicates the direction of transcription. Color coding: Black – ORFs for which products a functional assignment could be made based on the known functions of homologous proteins; Gray - ORFs coding for products that are similar to proteins of unknown function; Empty – ORFs coding for proteins that share no significant homology with proteins in available databases. Functionally assigned ORFs are also listed in the figure. The information in the figure is also included in tabular form in FIG. 4.

[0052] FIGS. 4A-4K: Features of the bacteriophage F394/08 genome, including gene products and assignment of putative functions. The figure includes a listing of the ORFs of the genome and provides for each ORF (i) its position within the genome, (ii) the encoded amino acid

sequence, (iii) a listing of homologous proteins and conserved domains within its encoded polypeptide and (iv) an assignment of putative function. ORFs 1-47 listed in FIG. 4 encode the amino acid sequences of SEQ ID NO:177-223, respectively.

[0053] FIGS. 5A-5B: Schematic of the organization of the F488/08 genome, comprising the nucleic acid sequence of SEQ ID NO:3. The open reading frames ("ORFs") predicted in the approximately 167kb genome are represented by arrows and numbered in black. The direction of an arrow indicates the direction of transcription. Color coding: Black – ORFs for which products a functional assignment could be made based on the known functions of homologous proteins; Gray - ORFs coding for products that are similar to proteins of unknown function; Empty – ORFs coding for proteins that share no significant homology with proteins in available databases. Functionally assigned ORFs are also listed in the figure. The information in the figure is also included in tabular form in FIG. 6.

[0054] FIGS. 6A-6DDD: Features of the bacteriophage F488/08 genome, including gene products and assignment of putative functions. The figure includes a listing of the ORFs of the genome and provides for each ORF (i) its position within the genome, (ii) the encoded amino acid sequence, (iii) a listing of homologous proteins, and conserved domains within its encoded polypeptide, and (iv) an assignment of putative function. ORFs 1-283 listed in FIG. 6 encode the amino acid sequences of SEQ ID NO:224-506, respectively.

[0055] FIG. 7: Schematic of the organization of the F510/08 genome, comprising the nucleic acid sequence of SEQ ID NO:4. The open reading frames ("ORFs") predicted in the approximately 43kb genome are represented by arrows and numbered in black. The direction of an arrow indicates the direction of transcription. Color coding: Black – ORFs for which products a functional assignment could be made based on the known functions of homologous proteins; Gray - ORFs coding for products that are similar to proteins of unknown function; Empty – ORFs coding for proteins that share no significant homology with proteins in available databases. Functionally assigned ORFs are also listed in the figure. The information in the figure is also included in tabular form in FIG. 8.

[0056] FIGS. 8 A-8S: Features of the bacteriophage F510/08 genome, including gene products and assignment of putative functions. The figure includes a listing of the ORFs of the genome and provides for each ORF (i) its position within the genome, (ii) the encoded amino acid sequence, (iii) a listing of homologous proteins, and conserved domains within its encoded

polypeptide, and (iv) an assignment of putative function. ORFs 1-53 listed in FIG. 8 encode the amino acid sequences of SEQ ID NO:507-559, respectively.

[0057] FIG. 9: Schematic of the organization of the F44/10 genome, comprising the nucleic acid sequence of SEQ ID NO:560. The open reading frames ("ORFs") predicted in the approximately 137kb genome are represented by arrows and numbered in black. The direction of an arrow indicates the direction of transcription. Color coding: Black – ORFs for which products a functional assignment could be made based on the known functions of homologous proteins; Gray - ORFs coding for products that are similar to proteins of unknown function; Empty – ORFs coding for proteins that share no significant homology with proteins in available databases. Functionally assigned ORFs are also listed in the figure. The information in the figure is also included in tabular form in FIG. 10.

[0058] FIGS. 10A-10QQ: Features of the bacteriophage F44/10 genome, including gene products and assignment of putative functions. The figure includes a listing of the ORFs of the genome and provides for each ORF (i) its position within the genome, (ii) the encoded amino acid sequence, (iii) a listing of homologous proteins and conserved domains within its encoded polypeptide and (iv) an assignment of putative function. ORFs 1-216, including ORFs 1a, 1b, 82a, 82b, 82c, 114a, and 114b, listed in FIG. 10 encode the amino acid sequences of SEQ ID NO:561-780, respectively.

[0059] FIG. 11: Schematic of the organization of the F387/08 genome, comprising the nucleic acid sequence of SEQ ID NO:781. The open reading frames ("ORFs") predicted in the approximately 167 kb genome are represented by arrows and numbered in black. The direction of an arrow indicates the direction of transcription. Color coding: Black – ORFs for which products a functional assignment could be made based on the known functions of homologous proteins; Gray - ORFs coding for products that are similar to proteins of unknown function; Empty – ORFs coding for proteins that share no significant homology with proteins in available databases. Functionally assigned ORFs are also listed in the figure. The information in the figure is also included in tabular form in FIG. 12.

[0060] FIGS. 12A-12UUU: Features of the bacteriophage F387/08 genome, including gene products and assignment of putative functions. The figure includes a listing of the ORFs of the genome and provides for each ORF (i) its position within the genome, (ii) the encoded amino acid sequence, (iii) a listing of homologous proteins and conserved domains within its encoded

polypeptide and (iv) an assignment of putative function. ORFs 1-292 listed in FIG. 12 encode the amino acid sequences of SEQ ID NOs: 782-1073, respectively.

[0061] FIGS. 13A-13B: Schematic of the organization of the F125/10 genome, comprising the nucleic acid sequence of SEQ ID NO:1074. The open reading frames ("ORFs") predicted in the approximately 145kb genome are represented by arrows and numbered in black. The direction of an arrow indicates the direction of transcription. Color coding: Black – ORFs for which products a functional assignment could be made based on the known functions of homologous proteins; Gray - ORFs coding for products that are similar to proteins of unknown function; Empty – ORFs coding for proteins that share no significant homology with proteins in available databases. Functionally assigned ORFs are also listed in the figure. The information in the figure is also included in tabular form in FIGS. 14A-14ZZZ

[0062] FIGS. 14A-14ZZZ: Features of the bacteriophage F125/10 genome, including gene products and assignment of putative functions. The figure includes a listing of the ORFs of the genome and provides for each ORF (i) its position within the genome, (ii) the encoded amino acid sequence, (iii) a listing of homologous proteins and conserved domains within its encoded polypeptide and (iv) an assignment of putative function. ORFs 1b-221, 1a listed in this Figure encode the amino acid sequences of SEQ ID NO:1075-1300, respectively, including 36a and 36b, 68a and 68b, and 153a and 153b.

[0063] FIGS. 15A-15III: The nucleotide sequence of the genome of bacteriophage F391/08 (SEQ ID NO:1).

[0064] FIGS. 16A-16Q: The nucleotide sequence of the genome of bacteriophage F394/08 (SEQ ID NO:2).

[0065] FIGS. 17A-17KKKK: The nucleotide sequence of the genome of bacteriophage F488/08 (SEQ ID NO:3).

[0066] FIGS. 18A-18X: The nucleotide sequence of the genome of bacteriophage F510/08 (SEQ ID NO:4).

[0067] FIGS. 19A-19UUU: The nucleotide sequence of the genome of bacteriophage F44/10 (SEQ ID NO:560).

[0068] FIGS. 20A-20KKKK: The nucleotide sequence of the genome of bacteriophage F387/08 (SEQ ID NO:781).

[0069] FIGS. 21A-21ZZZ: The nucleotide sequence of the genome of bacteriophage F125/10 (SEQ ID NO:1074).

6.1 DETAILED DESCRIPTION

[0070] The present invention is directed to isolated bacteriophage, and their isolated polypeptide products, having antibacterial activity against one or more species or strains of the nosocomial pathogens *Klebsiella pneumoniae, Acinetobacter baumannii, Escherichia coli, Pseudomonas aeruginosa,* and *S. aureus.* In one embodiment, isolated bacteriophage or polypeptides are provided that exhibit antimicrobial and/or antibacterial activity against methicillin-resistant strains of *S. aureus* (MRSA). In addition, the bacteriophage and polypeptides of the invention may exhibit antibacterial or antimicrobial activity against one or more species or strains of pathogenic bacteria including, but not limited to, S. *epidermidis, S. auricularis, S. capitis, S. haemolyticus, S. hominis, S. saprophyticus, S. simulans, S. xylosis, Micrococcus luteus, Bacilus subtilis, B. pumilus, E. hirae* and *E. avium.*

[0071] In some embodiments, the invention provides a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 1. A specific example in accordance with this embodiment is the isolated bacteriophage F391/08, which targets a number of strains of *Klebsiella* species, including *K. pneumoniae* and *K. oxytoca.* A schematic organization of the F391/08 genome, comprising the nucleic acid sequence of SEQ ID NO: 1, is provided in FIG. 1. Open reading frames (ORFs) in the F391/08 genome are provided in FIG 2. Also provided are the positions of the ORFs within the genome, the amino acid sequences encoded by the ORFs, homologous or similar proteins and conserved domains within the encoded polypeptide, and the assignment of putative functions. ORFs 1-172 listed in FIG. 2 encode the amino acid sequences of SEQ ID NOs: 5-176, respectively.

[0072] In some embodiments, the invention provides a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 781. A specific example in accordance with this embodiment is the isolated bacteriophage F387/08, which targets a number of strains of *Klebsiella* species, including *K. pneumoniae* and *K. oxytoca.* A schematic organization of the F387/08 genome, comprising the nucleic acid sequence of SEQ ID NO: 781, is provided in FIG. 11. Open reading frames (ORFs) in the F387/08 genome are provided in FIG 12. Also provided are the positions of the ORFs within the genome, the amino acid sequences

encoded by the ORFs, homologous or similar proteins and conserved domains within the encoded polypeptide, and the assignment of putative functions. ORFs 1-292 listed in FIG. 12 encode the amino acid sequences of SEQ ID NOs: 782-1073, respectively.

[0073] *Klebsiella pneumoniae* is a Gram-negative, non-motile, rod-shaped bacterium, found in the normal flora of the mouth, skin, and intestines. As an encapsulated, facultative anaerobe, the bacterium also naturally occurs in the soil and about 30% of strains can fix nitrogen in anaerobic conditions. Clinically, it is the most important member of the *Klebsiella* genus of *Enterobacteriaceae*, and also is closely related to *K. oxytoca*. *Klebsiella* infections tend to occur in people with a weakened immune system from improper diet, *e.g.* in alcoholics and diabetics. *Klebsiella* is also an opportunistic pathogen for patients with chronic pulmonary disease, enteric pathogenicity, nasal mucosa atrophy, and rhinoscleroma. New antibiotic resistant strains of *K. pneumoniae* are appearing, and it is increasingly found as a nosocomial infection, for example, due to contact with contaminated instruments.

[0074] In some embodiments, the invention provides a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 2. A specific example in accordance with this embodiment is the isolated bacteriophage F394/08, which targets a number of strains of *Acinetobacter* species, including *A. baumanni*, *A. calcoaceticus*, and *A. Iwoffi*. A schematic organization of the F394/08 genome, comprising the nucleic acid sequence of SEQ ID NO: 2, is provided in FIG. 3. Open reading frames (ORFs) in the F394/08 genome are provided in FIG 4. Also provided are the positions of the ORFs within the genome, the amino acid sequences encoded by the ORFs, homologous or similar proteins and conserved domains within the encoded polypeptide, and the assignment of putative functions. ORFs 1-47 listed in FIG. 4 encode the amino acid sequences of SEQ ID NOs: 177-223, respectively.

[0075] *Acinetobacter baumannii* is a species of bacteria that causes a number of severe clinical infections, particularly in individuals with compromised immune systems. *A. baumannii* is a pleomorphic aerobic gram-negative bacillus that is commonly isolated from the hospital environment and from hospitalized patients. The bacterium often enters the body open wounds, catheters, or breathing tubes. *A. baumannii* usually colonizes aquatic environments and is often cultured from hospitalized patients' sputum or respiratory secretions, wounds, and urine. In a hospital setting, *A. baumannii* commonly colonizes irrigating solutions and intravenous

solutions. It is also known to be resistant to multiple antibiotics and the number of nosocomial infections caused by *A. baumanni* has increased in recent years.

[0076] In some embodiments, the invention provides a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 3. A specific example in accordance with this embodiment is the isolated bacteriophage F488/08, which targets a number of strains of *Escherichia* species, including *E. coli*. A schematic organization of the F488/08 genome, comprising the nucleic acid sequence of SEQ ID NO: 3, is provided in FIG. 5. Open reading frames (ORFs) in the F488/08 genome are provided in FIG 6. Also provided are the positions of the ORFs within the genome, the amino acid sequences encoded by the ORFs, homologous or similar proteins and conserved domains within the encoded polypeptide, and the assignment of putative functions. ORFs 1-283 listed in FIG. 6 encode the amino acid sequences of SEQ ID NOs: 224-506, respectively.

[0077] *Escherichia coli* is a Gram negative rod-shaped bacterium that is commonly found in the lower intestine of mammals, comprising the primaru facultative anaerobic of the human gastrointestinal tract. Most *E. coli* strains are harmless and may form part of the normal flora of the gut, where they may benefit their hosts, *e.g.*, by producing vitamin K2 and/or by preventing the establishment of pathogenic bacteria within the intestines. Certain virulent strains of *E. coli*, however, may cause food poisoning, typically manifesting as a bout of diarrhea. More virulent strains, such as O157:H7, can cause serious illness and even death in the elderly, the very young, or the immunocompromised. Strains such as O157:H7, as well as O121 and O104:H21, produce potentially lethal toxins. Virulent strains of *E. coli* also can cause gastroenteritis, urinary tract infections, and neonatal meningitis, as well as, in rarer cases, haemolytic-uremic syndrome (HUS), peritonitis, mastitis, septicemia, and Gram-negative pneumonia. Further, if *E. coli* bacteria escape the intestinal tract through a perforation (for example from a ruptured appendix, and ulcer, or a surgical error) and enter the abdomen, they usually cause peritonitis that can be fatal without prompt treatment. Intestinal mucosa-associated *E. coli* also are observed in increased numbers in the inflammatory bowel diseases, Crohn's disease and ulcerative colitis.

[0078] Antibiotics that may be used to treat *E. coli* infection include amoxicillin as well as other semi-synthetic penicillins, many cephalosporins, carbapenems, aztreonam, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin, and the aminoglycosides. Nonetheless, as Gram-negative organisms, *E. coli* are resistant to many antibiotics that are effective against

Gram-positive organisms and antibiotic resistance is a growing problem. Resistance to beta-lactam antibiotics, for example, has become a particular problem in recent decades, as strains of bacteria that produce extended-spectrum beta-lactamases become more common. These beta-lactamase enzymes can render many, if not all, penicillins and/or cephalosporins therapeutically ineffective. Extended-spectrum beta-lactamase producing *E. coli* strains that are resistant to an array of antibiotics result in infections that are particularly difficult to treat.

[0079] In some embodiments, the invention provides a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 4. A specific example in accordance with this embodiment is the isolated bacteriophage F510/08, which targets a number of strains of *Pseudomonas* species, including *P. aeruginosa*. A schematic organization of the F510/08 genome, comprising the nucleic acid sequence of SEQ ID NO: 4, is provided in FIG. 7. Open reading frames (ORFs) in the F510/08 genome are provided in FIG 8. Also provided are the positions of the ORFs within the genome, the amino acid sequences encoded by the ORFs, homologous or similar proteins and conserved domains within the encoded polypeptide, and the assignment of putative functions. ORFs 1-53 listed in FIG. 8 encode the amino acid sequences of SEQ ID NOs: 507-559, respectively.

[0080] *Pseudomonas aeruginosa* is a common Gram-negative rod-shaped bacterium found in soil, water, skin flora and most man-made environments. It thrives not only in normal atmospheres, but also with little oxygen as a facultative anaerobe, and can infect damaged tissues or immunocomromised inidviduals. When such colonisations occur in critical body organs such as the lungs, the urinary tract, and kidneys, the results can be fatal. Because it thrives on surfaces, this bacterium is also found on and in medical equipment including catheters, causing cross infections in hospitals and clinics. *P. aeruginosa* is one of the most relevant opportunistic, nosocomial pathogens, and it has been estimated that one in ten hosptical-acquired infections are from *Pseudomonas*. *P. aeruginosa* is also the most common cause of burn injury infections and the most frequent colonizer of medical devices, such as catheters.

[0081] In some embodiments, the invention provides a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 560. A specific example in accordance with this embodiment is the isolated bacteriophage F44/10, which targets a number of strains of *Staphylococcus* species, including *S. aureus*. A schematic organization of the F44/10 genome, comprising the nucleic acid sequence of SEQ ID NO: 560, is provided in

FIG. 9. Open reading frames (ORFs) in the F44/10 genome are provided in FIG 10. Also provided are the positions of the ORFs within the genome, the amino acid sequences encoded by the ORFs, homologous or similar proteins and conserved domains within the encoded polypeptide, and the assignment of putative functions. ORFs 1-216, including 1a, 1b, 82a, 82b, 82c, 114a, and114b, listed in FIG. 10, encode the amino acid sequences of SEQ ID NOs: 561-780, as indicated in the Figure.

[0082] In some embodiments, the invention provides a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 1074. A specific example in accordance with this embodiment is the isolated bacteriophage F125/10, which targets a number of strains of *Staphylococcus* species, including *S. aureus*. A schematic organization of the F125/10 genome, comprising the nucleic acid sequence of SEQ ID NO: 1074, is provided in FIG. 13. Open reading frames (ORFs) in the F125/10 genome are provided in FIG 14. Also provided are the positions of the ORFs within the genome, the amino acid sequences encoded by the ORFs, homologous or similar proteins and conserved domains within the encoded polypeptide, and the assignment of putative functions. ORFs 1-221, including 1a, 1b, 36a, 36b, 68a, 68b, 153a, and 153b, listed in FIG. 14, encode the amino acid sequences of SEQ ID NOs: 1075-1300, as indicated in the Figure.

[0083] *Staphylococcus aureus* is a Gram-positive spherical facultative anaerobe, which grows as grape-like clusters with a characteristic golden color, and the most common cause of staph infections. It is frequently part of the flora of human skin and responsible for a range of infections, including pimples, carbuncles, scalded skin syndrome, pneumonia, gastroenteritis, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteremia, and sepsis. It remains one of the five most common causes of nosocomial infections, often causing post-surgical wound infections. It has been estimated that about 50,000 patients in American hospitals contract a staph infection. Of particular concern are the methicillin-resistant *Staphylococcus aureus* strains (MRSA). MRSA remained an uncommon occurrence in hospital setting until the 1990's, when there was an explosion in MRSA prevalence in hospitals, where it now is considered endemic, especially in the UK. Johnson A.P., et al., *J. Antimicrobial Chemotherapy*, 48(1): 143-144 (2001). *S. aureus* has proven to be a very hardy bacterium, and was shown in one study that it could survive on polyester for almost three months, polyester being the main

material used in hospital privacy curtains. Neely, A.N., et al., J. Clin. Microbiol., 38(2): 724-726 (2000).

[0084] The following organisms were deposited on September 16, 20011, with NCIMB Limited, located at the Ferguson Building, Craibstone Estate, Bucksburn, Aberdeen, AB21 9YA, Scotland UK, under the provisions of the Budapest Treaty for the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure ("Budapest Treaty") and the NCIMB has assigned the corresponding NCIMB accession numbers as follows: host strain *Pseudomonas aeruginosa* 433/07 B2, NCIMB 41861; host strain *Staphylococcus aureus* 743/06 B1, NCIMB 41862; host strain *Acinetobacter baumannii* 1305/05 B3, NCIMB 41863; *Pseudomonas aeruginosa* phage F770/05, NCIMB 41864; *Acinetobacter baumannii* phage F1245/05, NCIMB 41865; *Staphylococcus aureus* phage F125/10, NCIMB 41866; *Staphylococcus aureus* phage F44/10, NCIMB 41867; and *Pseudomonas aeruginosa* phage F510/08, NCIMB 41868, all of which are incorporated by reference herein.

[0085] In certain embodiments, the bacteriophage of the invention comprises or consists of a genome having a sequence identity of at least 85%, 90%, 95%, 96%, 97%, 98% or at least 99% with the nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 560, SEQ ID NO: 781, or SEQ ID NO: 1074, which bacteriophage exhibits at least one biological activity, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), of one or more of bacteriophage F391/08, F394/08, F488/08, F510/08, F387/08, FF44/10, and F125/10. Alternatively or in addition, the bacteriophage of the invention may have a genome comprising a functional fragment of the nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO:4, SEQ ID NO: 560, SEQ ID NO: 781, or SEQ ID NO: 1074, including the sequences of any of the open reading frames described in FIGS. 2, 4, 6, 8, 10, 12, and/or 14.

[0086] The invention also provides for isolated bacteria infected with one or more of the bacteriophage of the invention. In certain embodiments, the invention provides isolated *K. pneumoniae* infected with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 1 and/or SEQ ID NO: 781. In certain embodiments, the invention provides isolated *A. baumannii* infected with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 2. In certain embodiments, the invention provides isolated *E. coli* infected with a bacteriophage having a

genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 3. In certain embodiments, the invention provides isolated *P. aeruginosa* infected with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 4. In certain embodiments, the invention provides isolated *S. aureus* infected with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 560 and/or SEQ ID NO: 1074.

[0087] The invention provides for methods of production and isolation of a bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 560, SEQ ID NO: 781, or SEQ ID NO: 1074. In certain embodiments, the invention provides for a method of producing and/or isolating a bacteriophage having a genome that comprises or consists of the nucleic acid sequence of SEQ ID NO: 1 and/or SEQ ID NO: 781 comprising (i) obtaining a culture of *K. pneumoniae*, (ii) infecting it with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 1 and/or SEQ ID NO: 781; (iii) culturing until significant lysis of the culture is observed; and (iv) isolating from the culture the bacteriophage. In other embodiments, the invention provides for a method of producing and/or isolating a bacteriophage having a genome that comprises or consists of the nucleic acid sequence of SEQ ID NO: 2 comprising (i) obtaining a culture of *A. baumannii*, (ii) infecting it with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 2; (iii) culturing until significant lysis of the culture is observed; and (iv) isolating from the culture the bacteriophage. In still other embodiments, the invention provides for a method of producing and/or isolating a bacteriophage having a genome that comprises or consists of the nucleic acid sequence of SEQ ID NO: 3 comprising (i) obtaining a culture of *E. coli*, (ii) infecting it with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence of ID NO: 3; (iii) culturing until significant lysis of the culture is observed; and (iv) isolating from the culture the bacteriophage. In yet still other embodiments, the invention provides for a method of producing and/or isolating a bacteriophage having a genome that comprises or consists of the nucleic acid sequence of SEQ ID NO: 4 comprising (i) obtaining a culture of *P. aeruginosa*, (ii) infecting it with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 4; (iii) culturing until significant lysis of the culture is observed; and (iv) isolating from the culture the bacteriophage.

[0088] In yet still further embodiments, the invention provides for a method of producing and/or isolating a bacteriophage having a genome that comprises or consists of the nucleic acid sequence of SEQ ID NO: 560 and/or SEQ ID NO: 1074 comprising (i) obtaining a culture of *S. aureus*, (ii) infecting it with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 560 and/or SEQ ID NO: 1074; (iii) culturing until significant lysis of the culture is observed; and (iv) isolating from the culture the bacteriophage.

[0089] Bacteriophage may be isolated from a bacterial sample using any method described herein or known in the art (see, *e.g.*, Carlson, "Working with bacteriophage: common techniques and methodological approaches," *In,* Kutter and Sulakvelidze (Eds) Bacteriophage: Biology and Applications, 5th ed. CRC Press (2005); incorporated herein by reference in its entirety).

[0090] The invention also provides for polypeptides isolated from bacteriophage of the invention. The isolated polypeptides may be full length bacteriophage proteins or may be fragments, variants or derivatives of the bacteriophage proteins provided that the fragment, variant or derivative exhibit at least one biological activity associated with the bacteriophage or polypeptide from which it is derived. In certain embodiments, the polypeptides of the invention are isolated from bacteriophage F387/08 or F391/08 (which typically infect *K. pneumoniae*), F394/08 (which typically infects *A. baumannii*), bacteriophage F488/08 (which typically infects *E. coli*), bacteriophage F510/08 (which typically infects *P. aeruginosa*) or bacteriophage F44/10 or F125/40 (which typically infects *S. aureus*).

[0091] In specific embodiments, the polypeptide of the invention is a lysin isolated from a bacteriophage having a genome comprising or consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 560, SEQ ID NO: 781, or SEQ ID NO: 1074 (*e.g.*, bacteriophage F391/08, F394/08, F488/08, F510/08, F44/10, F387/08, or F125/10, respectively). In specific embodiments, the polypeptide of the invention is a lysin, *e.g.*, an endolysin or tail lysin, having the amino acid sequence comprising or consisting of SEQ ID NO: 20, SEQ ID NO: 80, SEQ ID NO: 192, SEQ ID NO: 282, SEQ ID NO: 547, SEQ ID NO: 556, SEQ ID NO: 557, SEQ ID NO: 598, SEQ ID NO: 1216, or SEQ ID NO: 1261. Predicted functions of said lysins include, for example an Ig-like virion protein (SEQ ID NO: 20), cell wall hydrdolase (SEQ ID NO: 80), endolysin; N-acetylmuramoyl-L-alanine amidase (SEQ ID NO: 192), soluble lysozyme (SEQ ID NO: 282), T4-like lysozyme (SEQ ID NO: 547), endolysin (SEQ ID NO: 556), lambda

Rz1-like protein (SEQ ID NO: 557), endolysin (SEQ ID NO: 598), endolysin (SEQ ID NO:1216), and tail lysin (SEQ ID NO: 1261).

[0092] In other embodiments, the isolated polypeptide of the invention is a fragment, variant or derivative of an endolysin or lysin isolated from a bacteriophage of the invention, which fragment, variant or derivative exhibits at least one biological activity, preferably antibacterial activity (*e.g.*, lytic killing activity), of the endolysin, lysin or bacteriophage from which it is isolated or derived. Accordingly, in certain embodiments, the invention provides isolated polypeptides that are fragments, variants or derivatives of endolysins or lysins isolated from bacteriophage of the invention, which fragments, variants or derivatives exhibit antibacterial or antimicrobial activity (*e.g.*, lytic killing activity) against one or more of *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, or *S. aureus*. In other embodiments, the isolated polypeptides are fragments, variants or derivatives of endolysins or lysins isolated from bacteriophage of the invention that exhibit antibacterial or antimicrobial activity (*e.g.*, lytic killing activity) against one or more species of bacteria other than *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, or *S. aureus*. In certain embodiments, the polypeptide of the invention comprises or consists of the amino acid sequence SEQ ID NO: 20 and/or SEQ ID NO: 80, or a fragment, variant or derivative thereof, which polypeptide exhibits antibacterial or antimicrobial activity against one or more strains of *K. pneumoniae*, *e.g.*, against the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 1. In other embodiments, the polypeptide of the invention comprises or consists of the amino acid sequence SEQ ID NO: 192, or a fragment, variant or derivative thereof, which polypeptide exhibits antibacterial or antimicrobial activity against one or more strains of *A. baumannii*, *e.g.*, against the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 2. In yet still other embodiments, the polypeptide of the invention comprises or consists of the amino acid sequence SEQ ID NO: 282, or a fragment, variant or derivative thereof, which polypeptide exhibits antibacterial or antimicrobial activity against one or more strains of *E. coli*, *e.g.*, against the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 3. In yet still other embodiments, the polypeptide of the invention comprises or consists of the amino acid sequence SEQ ID NO: 547, SEQ ID NO: 556, SEQ ID NO: 557, or a fragment, variant or derivative thereof, which polypeptide exhibits antibacterial or antimicrobial activity against one or more strains of *P. aeruginosa*, *e.g.*, against the bacteriophage having a genome

comprising or consisting of the nucleic acid sequence SEQ ID NO: 4. In yet still further embodiments, the polypeptide of the invention comprises or consists of the amino acid sequence SEQ ID NO: 598, or a fragment, variant or derivative thereof, which polypeptide exhibits antibacterial or antimicrobial activity against one or more strains of *S. aureus*, *e.g.*, against the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 560. In yet still further embodiments, the polypeptide of the invention comprises or consists of the amino acid sequence SEQ ID NO: 1216 and/or SEQ ID NO: 1261, or a fragment, variant or derivative thereof, which polypeptide exhibits antibacterial or antimicrobial activity against one or more strains of *S. aureus*, *e.g.*, against the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 1074.

[0093] In certain embodiments, the polypeptide of the invention comprises or consists of a CHAP domain isolated from an endolysin or lysin of bacteriophage F387/08, F391/08, F394/08, F488/08, F510/08, F44/10, or F125/10. Isolated CHAP domains have been demonstrated to retain the antibacterial activity, *e.g.*, lytic killing activity, of the endolysin or lysin from which they are derived; CHAP domains may be identified and isolated by methods routine in the art (see, *e.g.*, Rigden et al., 2003, *Trends Biochem. Sci.* 28:230-234; Bateman et al., 2003, *Trends Biochem. Sci.* 28:234-237, each of which is incorporated by reference herein in its entirety). In specific embodiments, the polypeptide of the invention comprises or consists of a CHAP domain isolated from a polypeptide having an amino acid sequence of SEQ ID NO: 20, SEQ ID NO: 80, SEQ ID NO: 192, SEQ ID NO: 282, SEQ ID NO: 547, SEQ ID NO: 556, SEQ ID NO: 557, SEQ ID NO: 598, SEQ ID NO: 1216, or SEQ ID NO: 1261. In other embodiments the invention provides for a fragment, variant or derivative of a CHAP domain of isolated from an endolysin or lysin of bacteriophage bacteriophage F387/08, F391/08, F394/08, F488/08, F510/08, F44/10, F125/10, which fragment, variant, or derivative exhibits at least one biological activity, *e.g.*, lytic cell killing, of the CHAP domain from which it was derived.

[0094] In certain embodiments, a polypeptide of the invention comprises or consists of a tail protein (*e.g.*, tail component, tail fiber protein, adsorption associated tail protein, tail length tape measure protein, baseplate wedge subunit), or fragment, variant, or derivative thereof, isolated from a bacteriophage having a genome comprising or consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO:4, SEQ ID NO: 560, SEQ ID NO: 781, or SEQ ID NO: 1074 (*e.g.*, bacteriophage F391/08, F394/08, F488/08, F510/08, F44/10, F387/08, or F125/10

respectively), wherein the tail protein, or fragment, variant, or derivative thereof has a biological function associated with the bacteriophage from which it is derived, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity). In specific embodiments, the antimicrobial or antibacterial activity of the tail protein is directed against at least one or more species or strains of *K. pneumoniae, A. baumannii, E. coli, P. aeruginosa,* and *S. aureus*. In specific embodiments, the polypeptide of the invention is a tail protein having the amino acid sequence comprising or consisting of SEQ ID NO: 15, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 629; SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, SEQ ID NOs: 1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1217, SEQ ID NO: 1250, or SEQ ID NO: 1266. In other embodiments, the isolated polypeptide of the invention is a fragment, variant or derivative of the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 629; SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, SEQ ID NOs: 1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1217, SEQ ID NO: 1250, or SEQ ID NO: 1266, which fragment, variant, or derivative exhibits at least one biological activity or function of the bacteriophage from which it is isolated or derived, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity). In preferred embodiments, the at least one biological activity or function of the fragment, variant or derivative is directed against one or more strains of *K. pneumoniae, A. baumannii, E. coli, P. aeruginosa,* and *S. aureus.*

[0095] Predicted functions of said tail proteins include, for example a receptor-binding tail protein (SEQ ID NO: 15), major tail protein (SEQ ID NO: 26), minor tail protein (SEQ ID NO: 27), pore-forming tail tip protein (SEQ ID NO: 30), tail protein (SEQ ID NOs: 32-33), minor tail protein (SEQ ID NO: 34), phage tail protein (SEQ ID NO: 35), tail sheath protein (SEQ ID NO:

180), tail tape measure protein (SEQ ID NO: 183), tail protein (SEQ ID NO: 185), tail-fiber protein (SEQ ID NO: 190), tail tube protein (SEQ ID NO: 231), tail sheath monomer (SEQ ID NO: 232), tail sheath stabilizer and completion protein (SEQ ID NO:235), short tail fibers (SEQ ID NO: 239), base plate wedge completion tail pin (SEQ ID NOs: 240-241), base plate wedge completion tail fiber socket (SEQ ID NO: 242), base plate wedge subunit (SEQ ID NO: 243), base plate wedge initiator (SEQ ID NO: 244), base plate wedge (SEQ ID NO: 245), base plate hub subunit and tail lysozyme, cell-puncturing device (SEQ ID NO: 248), base plate wedge completion (SEQ ID NO: 249), tail completion and sheath stabilizer protein (SEQ ID NO: 252), chaperone long and short tail fiber assembly (SEQ ID NO: 254), tail fiber protein (SEQ ID NO: 433), tail fiber protein (SEQ ID NO: 434), hinge connecter long tail fiber (SEQ ID NO: 435), tail fiber hinge (SEQ ID NO: 436), proximal tail fiber subunit (SEQ ID NO: 437), base plate-tail tube initiatior (SEQ ID NO: 489), base plate (SEQ ID NO: 490), baseplate hub subunit, tail length determinator (SEQ ID NO: 491), base plate distal hub subunit (SEQ ID NO: 492), base plate hub subunit (SEQ ID NO: 493), base plate hub assembly catalyst (SEQ ID NO: 494), baseplate hub subunit (SEQ ID NO: 495), baseplate wedge subunit (SEQ ID NO: 496), tail tubular protein (SEQ ID NOs: 544-545), tail fiber protein (SEQ ID NO: 549 and SEQ ID NO: 551), major tail sheath protein (SEQ ID NO: 629); major tail protein (SEQ ID NO: 686); tail tube protein (SEQ ID NO: 789); fibritin (SEQ ID NO: 796); short tail fibers (SEQ ID NO: 797); base plate wedge completion tail pin (SEQ ID NO: 798); base plate wedge subunit and tail pin (SEQ ID NO: 799); baseplate wedge tail fiber connector (SEQ ID NO: 800); baseplate hub subunit and lysozyme (SEQ ID NO: 806); lysozyme (SEQ ID NO: 854); holin (SEQ ID NO: 999); distal long tail fiber assembly catalyst (SEQ ID NO: 1000); L-shaped tail fiber protein (SEQ ID NO: 1001); hinge connector of long tail fiber distal connector (SEQ ID NO: 1002); hinge connector of long tail fiber proximal connector (SEQ ID NO: 1003); long tail fiber proximal subnit (SEQ ID NO: 1004); baseplate tail tube initiator (SEQ ID NO: 1053); baseplate tail tube cap (SEQ ID NO: 1054); baseplate hub subunit, tail length determinator (SEQ ID NO: 1055); baseplate distal hub subunit (SEQ ID NO: 1056); baseplate hub subunit (SEQ ID NOs: 1057 and 1059); baseplate hub assemply catalyst (SEQ ID NO: 1058); baseplate wedge subunit (SEQ ID NO: 1060); major tail protein (SEQ ID NO: 1077); holin (SEQ ID NO: 1217); major tail sheath protein (SEQ ID NO: 1250); and baseplate protein (SEQ ID NO: 1266).

[0096] In certain embodiments, the invention encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, or SEQ ID NOs: 32-35, which exhibits a biological function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 1, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *K. pneumoniae*. In certain embodiments, the invention encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, or SEQ ID NOs: 1053-1060, which exhibits a biological function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 781, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *K. pneumoniae*. In other embodiments, the invention encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, or SEQ ID NO: 190, which exhibits a biological function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 2, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *A. baumannii*.

[0097] In certain embodiments, the invention encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NOs: 433-437, SEQ ID NOs: 489-495, or SEQ ID NO: 496, which exhibits a biological function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 3, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *E. coli*. In certain embodiments, the invention encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 549, or SEQ ID NO: 551, which exhibits a biological function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 4, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *P. aeruginosa*. In certain embodiments, the invention encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 629 or SEQ ID NO: 686, which exhibits a biological function

associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 560, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *S. aureus*. In certain embodiments, the invention encompasses a variant, fragment or derivative of the amino acid sequence of SEQ ID NO: 1077, SEQ ID NO: 1216, SEQ ID NO: 1217, SEQ ID NO: 1250, SEQ ID NO: 1261, or SEQ ID NO: 1266, which exhibits a biological function associated with the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 1074, *e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity), which function is directed against one or more strains of *S. aureus*.

[0098] In certain embodiments, the isolated polypeptide of the invention is a variant of a bacteriophage polypeptide, which variant comprises or consists of a amino acid sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or greater sequence identity to a second amino acid sequence of the same length (*i.e.*, consisting of the same number of residues), which second amino acid sequence is SEQ ID NO: 15, SEQ ID NO: 20, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, SEQ ID NO: 80, SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 192, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NO: 282, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 547, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 556, SEQ ID NO: 557, SEQ ID NO: 598, SEQ ID NO: 629, SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, SEQ ID NOs: 1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1216, SEQ ID NO: 1217, SEQ ID NO: 1250, SEQ ID NO: 1261, SEQ ID NO: 1266 and/or a fragment thereof, and wherein the variant exhibits at least one biological function or activity of the bacteriophage from which it was derived (*e.g.*, antimicrobial or antibacterial activity (*e.g.*, lytic killing activity)) against one or more strains of bacteria, *e.g.*, Gram-positive bacteria (*e.g.*, *S. aureus*), Gram-negative bacteria (*e.g.*, *K. pneumoniae, A baumannii, E. coli, P. aeruginosa*) or bacteria not classified as either Gram-positive or Gram-negative.

[0099] In certain embodiments, the invention provides an isolated polypeptide having an amino acid sequence of any of SEQ ID NOs: 5-176, SEQ ID NOs: 177-223, SEQ ID NOs: 224-506,

SEQ ID NOs: 507-559, SEQ ID NOs: 561-780, SEQ ID NOs: 782-1073, and SEQ ID NOs: 1075-1300 and active biologic fragments thereof. In preferred embodiments, the variant polypeptide of the invention exhibits at least one biologic activity associated with the polypeptide or bacteriophage from which it was isolated or derived, *e.g.*, lytic activity directed against at least one or more strains of *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*.

[00100] In other embodiments, the invention provides an isolated nucleic acid sequence encoding the amino acid sequence of one of SEQ ID NOs: 5-176, SEQ ID NOs: 177-223, SEQ ID NOs: 224-506, SEQ ID NOs: 507-559, SEQ ID NOs: 561-780, SEQ ID NOs: 782-1073, and SEQ ID NOs: 1075-1300 and active fragments thereof. In other embodiments the invention provides the nucleic acid sequence encoding any of the open reading frames identified in FIGS. 2, 4, 6, 8, 10, 12, and/or 14.

[00101] In certain embodiments, the polypeptides of the present invention are recombinantly fused or chemically conjugated (including both covalently and non-covalently conjugations) to therapeutic agents, *e.g.*, heterologous polypeptides or small molecules, to generate fusion proteins or chimeric polypeptides. The fusion does not necessarily need to be direct, but may occur through linker sequences or through chemical conjugation. Non-limiting examples of therapeutic agents to which the polypeptides of the invention may be conjugated are peptide or non-peptide cytotoxins (including antimicrobials and/or antibiotics), tracer/marker molecules (*e.g.*, radionuclides and fluorphores) and other antibiotic or antibacterial compounds known in the art.

6.2 ANTIBIOTIC COMPOSITIONS

[00102] The isolated bacteriophage or polypeptides of the present invention may be administered alone or incorporated into a pharmaceutical composition for the use in treatment or prophylaxis of bacterial infections, *e.g.*, infections caused by bacteria including, but not limited to, *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa* and *S. aureus*. The polypeptides may be combined with a pharmaceutically acceptable carrier, excipient, or stabilizer. Examples of pharmaceutically acceptable carriers, excipients and stabilizers include, but are not limited to, buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight polypeptides; proteins, such as serum albumin and gelatin; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine,

arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN™, polyethylene glycol (PEG), and PLURONICS™. The pharmaceutical compositions of the present invention (*e.g.*, antibacterial compositions) can also include a lubricant, a wetting agent, a sweetener, a flavoring agent, an emulsifier, a suspending agent, and a preservative, *e.g.*, in addition to the above ingredients.

[00103] The bacteriophage and/or polypeptides of the present invention may also be combined with one or more therapeutic and/or prophylactic agents useful for the treatment of bacterial infection as described herein and/or known in the art (*e.g.* one or more lysins). The pharmaceutical compositions of the invention may therefore comprise two or more isolated bacteriophage of the invention (with antibacterial activity against the same or different bacterial species or strains), the combination of a bacteriophage and a polypeptide of the invention or the combination of a bacteriophage and/or polypeptide of the invention and a bacteriophage and/or polypeptide known in the art. In specific embodiments, the therapeutic components of a combination target two or more species or strains of bacteria or exhibit differing enzymatic activity. For example, lysins in general exhibit one of amidase, endopeptidase, muramidase or glucosamidase activity. Accordingly, the combination of lysins exhibiting different activities may provide synergistic enhancement to the therapeutic activity of the pharmaceutical composition of the invention.

[00104] In some embodiments, a number of different bacteriophage are combined to provide a "phage cocktail." In some embodiments, the phage cocktail comprises at least 2 phage, at least 3 phage, at least 4 phage, at least 5 phage, at least 6 phage, at least 7 phage, at least 8 phage, at least 9 phage, at least 10 phage, or more. In some embodiments, the phage cocktail comprises 2-20 phage, 2-15 phage, 2-10 phage, 3-8 phage, or 4-6 phage.

[00105] In some embodiements, at least one phage of the cocktail is a phage with antibcateial activity against at least one Gram-negative bacteria, including but not limited to *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Escherichia coli*, and *Pseudomonas aeruginosa*; and/or against at least one Gram-positive bacteria including but not limited to *Staphylococcus aureus*. In certain embodiments, at least one phage of the cocktail is F391/08, having a genome comprising the nucleic acid sequence of SEQ ID NO:1 and exhibiting

antibacterial activity against one or more strains of *Klebsiella pneumoniae*. In certain embodiments, at least one phage of the cocktail is F394/08, having a genome comprising the nucleic acid sequence of SEQ ID NO:2 and exhibiting antibacterial activity against one or more strains of *Acinetobacter baumannii*. In certain embodiments, at least one phage of the cocktail is F488/08, having a genome comprising the nucleic acid sequence of SEQ ID NO:3 and exhibiting antibacterial activity against one or more strains of *Escherichia coli*. In certain embodiments, at least one phage of the cocktail is F510/08, having a genome comprising the nucleic acid sequence of SEQ ID NO:4 and exhibiting antibacterial activity against one or more strains of *Pseudomonas aeruginosa*. In certain embodiments, at least one phage of the cocktail is F44/10, having a genome comprising the nucleic acid sequence of SEQ ID NO:560 and exhibiting antibacterial activity against one or more strains of *Staphylococcus aureus*. In certain embodiments, at least one phage of the cocktail is F387/08, having a genome comprising the nucleic acid sequence of SEQ ID NO:781 and exhibiting antibacterial activity against one or more strains of *Klebsiella pneumoniae*. In certain embodiments, at least one phage of the cocktail is F125/10, having a genome comprising the nucleic acid sequence of SEQ ID NO:1074 and exhibiting antibacterial activity against one or more strains of *Staphylococcus aureus*.

[00106] In certain embodiments, at least one phage of the cocktail is F170/08, having a genome as disclosed in WO 2010/090542, and exhibiting antibacterial activity against one or more strains of *Enterococcus faecalis or faecium*. In certain embodiments, at least one phage of the cocktail is F168/08, having a genome as disclosed in WO 2010/090542, and exhibiting antibacterial activity against one or more strains of *Enterococcus faecalis or faecium*. In certain embodiments, at least one phage of the cocktail is F770/05, having a genome as disclosed in WO 2010/090542, and exhibiting antibacterial activity against one or more strains of *Pseudomonas aeruginos*. In certain embodiments, at least one phage of the cocktail is F1245/05, having a genome as disclosed in WO 2010/090542, and exhibiting antibacterial activity against one or more strains of *Acinetobacter baumannii*.

[00107] In some preferred embodiments, the cocktail comprises a phage having biological activity against *Acinetobacter*. For example, the cocktail may comprise F394/08 and/or F1245/05, exhibiting antibacterial activity against one or more strains of *Acinetobacter baumannii*. In certain embodiments, the phage cocktail comprises at least one phage exhibiting antibacterial activity against one or more strains of *Acinetobacter baumannii* and at least one

phage exhibiting antibacterial activity against a different bacteria. For example, in some embodiments, the phage cocktail comprises F394/08 and/or F1245/05 in combination with at least one other phage selected from F391/08, F488/08, F510/08, F44/10, F387/08, F170/08, F168/08, F770/05, and F125/10. In certain embodiments, the phage cocktail comprises at least one phage exhibiting antibacterial activity against one or more strains *Klebsiella pneumoniae* and at least one phage exhibiting antibacterial activity against a different bacteria. For example, in some embodiments, the phage cocktail comprises F391/08 and/or F387/08 in combination with at least one other phage selected from F394/08, F488/08, F510/08, F44/10, F1245/05, F170/08, F168/08, F770/05, and F125/10. In certain embodiments, the phage cocktail comprises at least one phage exhibiting antibacterial activity against one or more strains of *Escherichia coli* and at least one phage exhibiting antibacterial activity against a different bacteria. For example, in some embodiments, the phage cocktail comprises F488/08 in combination with at least one other phage selected from F391/08, F510/08, F44/10, F394/08, F387/08, F170/08, F168/08, F1245/05, F770/05, and F125/10.

[0108] In certain embodiments, the phage cocktail comprises at least one phage exhibiting antibacterial activity against one or more strains of *Pseudomonas aeruginosa* and at least one phage exhibiting antibacterial activity against a different bacteria. For example, in some embodiments, the phage cocktail comprises F510/08 and/or F770/05 in combination with at least one other phage selected from F391/08, F394/08, F488/08, F44/10, F387/08, F170/08, F168/08, F1245/05, and F125/10. In certain embodiments, the phage cocktail comprises at least one phage exhibiting antibacterial activity against one or more strains of *Staphylococcus aureus* and at least one phage exhibiting antibacterial activity against a different bacteria. For example, in some embodiments, the phage cocktail comprises F44/10 and/or F125/10 in combination with at least one other phage selected from F391/08, F394/08, F488/08, F510/08, F387/08, F170/08, F168/08, F770/05, and F1245/05. In certain embodiments, the phage cocktail comprises at least one phage exhibiting antibacterial activity against one or more strains of *Enterococcus faecalis or faecium* and at least one phage exhibiting antibacterial activity against a different bacteria. For example, in some embodiments, the phage cocktail comprises F170/08 and/or F168/08 in combination with at least one other phage selected from F391/08, F394/08, F488/08, F510/08, F44/10, F387/08, F770/05, F1245/05, and F125/10.

[0109] In certain embodiments, the phage cocktail comprises at least four (4) phage selected from the group consisting of F391/08, F394/08, F488/08, F510/08, F44/10, F387/08, F170/08, F168/08, F770/05, F1245/05, and F125/10. In certain embodiments, the phage cocktail comprises F391/08, F394/08, F488/08, and F510/08. In certain embodiments, the phage cocktail comprises F44/10, F387/08, F170/08, and F168/08. In certain embodiments, the phage cocktail comprises of F391/08, F394/08, F770/05, and F1245/05. In certain embodiments, the phage cocktail comprises F391/08, F394/08, F510/08, and/or F44/10. In certain embodiments, the phage cocktail comprises F391/08, F394/08, F44/10, and/or F387/08. In certain embodiments, the phage cocktail comprises F391/08, F394/08, F387/08, and/or F170/08. In certain embodiments, the phage cocktail comprises F391/08, F394/08, F170/08, and F168/08. In certain embodiments, the phage cocktail comprises F391/08, F394/08, F168/08, and/or F770/05. In certain embodiments, the phage cocktail comprises F391/08, F394/08, F770/05, and F1245/05.

[0110] In certain embodiments, the phage cocktail comprises F125/10, F391/08, F394/08, and F488/08. In certain embodiments, the phage cocktail comprises F125/10, F394/08, F488/08, and F510/08. In certain embodiments, the phage cocktail comprises F125/10, F488/08, F510/08, and

F44/10. In certain embodiments, the phage cocktail comprises F125/10, F44/10, F387/08, and F170/08. In certain embodiments, the phage cocktail comprises F125/10, F170/08, F168/08, and F770/05. In certain embodiments, the phage cocktail comprises F125/10, F770/05, F1245/05, and F391/08. In certain embodiments, the phage cocktail comprises F125/10, F510/08, F44/10, F387/08. In certain embodiments, the phage cocktail comprises F125/10, F387/08, F170/08, F168/08. In certain embodiments, the phage cocktail comprises F125/10, F168/08, F770/05, and F1245/05. In certain embodiments, the phage cocktail comprises F125/10, F1245/05, F391/08, and F394/08.

[0111] In certain embodiments, the phage cocktail comprises F394/08, F488/088, F510/08, and/or F44/10. In certain embodiments, the phage cocktail comprises F394/08, F488/088, F44/10, and/or F387/08. In certain embodiments, the phage cocktail comprises F394/08, F488/088, F387/08, and/or F170/08. In certain embodiments, the phage cocktail comprises F394/08, F488/088, F170/08, and/or F168/08. In certain embodiments, the phage cocktail comprises F394/08, F488/088, F168/08 and/or F770/05. In certain embodiments, the phage cocktail comprises F394/08, F488/088, FF770/05, and/or F1245/05. In certain embodiments, the phage cocktail comprises F394/08, F488/088, F1245/05 and/or F391/08. In certain embodiments, the phage cocktail comprises F488/08, F510/08, F44/10, and/or F387/08. In certain embodiments, the phage cocktail comprises F488/08, F510/08, F387/08, and/or F170/08. In certain embodiments, the phage cocktail comprises F488/08, F510/08, F170/08, and/or F168/08. In certain embodiments, the phage cocktail comprises F488/08, F510/08, F168/08, and/or F770/05. In certain embodiments, the phage cocktail comprises F488/08, F510/08, F770/05, and/or F1245/05. In certain embodiments, the phage cocktail comprises F488/08, F510/08, F1245/05, and/or F391/08. In certain embodiments, the phage cocktail comprises F488/08, F510/08, F391/08, and/or F394/08.

[0112] In certain embodiments, the phage cocktail comprises F387/08, F170/08, F168/08, and/or F770/05. In certain embodiments, the phage cocktail comprises F387/08, F170/08, F770/05, and/or F1245/05. In certain embodiments, the phage cocktail comprises F387/08, F170/08, F1245/05, and/or F391/08. In certain embodiments, the phage cocktail comprises F387/08, F170/08, F391/08, and/or F394/08. In certain embodiments, the phage cocktail comprises F387/08, F170/08, F394/08, and/or F488/08. In certain embodiments, the phage cocktail comprises F387/08, F170/08, F488/08, and/or F510/08. In certain embodiments, the phage

cocktail comprises F387/08, F170/08, F510/08, and/or F44/10. In certain embodiments, the phage cocktail comprises F387/08, F170/08, F44/10, and/or F387/08. In certain embodiments, the phage cocktail comprises F387/08, F170/08, F387/08, and/or F170/08. In certain embodiments, the phage cocktail comprises F387/08, F170/08, F170/08, and/or F168/08. In certain embodiments, the phage cocktail comprises F387/08, F170/08, F168/08, and/or F770/05. In certain embodiments, the phage cocktail comprises F387/08, F170/08, F770/05 and/or F1245/05. In certain embodiments, the phage cocktail comprises F387/08, F170/08, F1245/05, and/or F391/08. In certain embodiments, the phage cocktail comprises F387/08, F170/08, F391/08, and/or F394/08. In certain embodiments, the phage cocktail comprises F387/08, F170/08, F394/08 and/or F488/08. In certain embodiments, the phage cocktail comprises F387/08, F170/08, F488/08, and/or F510/08. In certain embodiments, the phage cocktail comprises F387/08, F170/08, F510/08, and/or F44/10.

[0113] In some embodiments, the phage cocktail comprises of F510/08, F44/10, F387/08, and/or F170/08. In some embodiments, the phage cocktail comprises F510/08, F44/10, F170/08, and/or F168/08. In some embodiments, the phage cocktail comprises F510/08, F44/10, F168/08, and/or F770/05. In some embodiments, the phage cocktail comprises F510/08, F44/10, F770/05, and/or F1245/05. In some embodiments, the phage cocktail comprises F510/08, F44/10, F1245/05, and/or F391/08. In some embodiments, the phage cocktail comprises F510/08, F44/10, F391/08, and/or F394/08. In some embodiments, the phage cocktail comprises F510/08, F44/10, F394/08, and/or F488/08.

[0114] In some embodiments, the phage comprises F44/10, F387/08, F170/08, and/or F168/08. In some embodiments, the phage comprises F44/10, F387/08, F168/08 and/or F770/05. In some embodiments, the phage comprises F44/10, F387/08, F770/05, and/or F1245/05. In some embodiments, the phage comprises F44/10, F387/08, F1245/05, and/or F391/08. In some embodiments, the phage comprises F44/10, F387/08, F391/08, and/or F394/08. In some embodiments, the phage comprises F44/10, F387/08, F394/08 and/or F488/08. In some embodiments, the phage comprises F44/10, F387/08, F488/08 and/or F510/08.

[0115] In some embodiments, the phage cocktail composition may or may not involve phage selected for increased *in vivo* half-life, *e.g.*, as disclosed in US 5,688,501, the contents of which are incorporated herein by reference.

[0116] In some embodiments, the cocktail comprises one or more polypeptides isolated from one or more phage, and/or a fragment, variant, or derivative thereof, in particular a polypeptide, fragment, variant, or derivative thereof which has antibacterial or antimicrobial activity. In some embodiments, the polypeptide, or fragment, variant, or derivative thereof comprises or consists of a lysin (or fragment thereof, *e.g.*, a CHAP domain) and/or a tail protein. In more specific embodiments, the polypeptide corresponds to an isolated polypeptide, fragment, variant, or derivative thereof, as described herein and/or in WO 2010/090542, the contents of which are incorporated by reference herein. In some embodiments, the cocktail is administered in the absence of an isolated polypeptide, such as in the absence of a lyase.

[0117] Other examples of other therapeutic agents that may be used in combination with the polypeptide of the invention include, but are not limited to, standard antibiotic agents, anti-inflammatory agents, antiviral agents, local anesthetic agents, and corticosteroids. In some embodiments, the cocktail is administered in the absence of an antibiotic.

[0118] Standard antibiotics that may be used with pharmaceutical compositions comprising a bacteriophage and/or polypeptide of the invention include, but are not limited to, amikacin, gentamicin, kanamycin, neomycin, netilmicin, paromomycin, rhodostreptomycin, streptomycin, tobramycin, apramycin, rifamycin, naphthomycin, mupirocin, geldanamycin, ansamitocin, carbacephems, imipenem, meropenem, ertapenem, faropenem, doripenem, panipenem/betamipron, biapenem, PZ-601, cephalosporins, cefacetrile, cefadroxil, cefalexin, cefaloglycin, cefalonium, cefaloridine, cefalotin, cefapirin, cefatrizine, cefazaflur, cefazedone, cefazolin, cefradine, cefroxadine, ceftezole, cefaclor, cefonicid, cefprozil, cefuroxime, cefuzonam, cefmetazole, cefotetan, cefoxitin, cefcapene, cefdaloxime, cefdinir, cefditoren, cefetamet, cefixime, cefmenoxime, cefteram, ceftibuten, ceftiofur, ceftiolene, ceftizoxime, ceftriaxone, cefoperazone, ceftazidime latamoxef, cefclidine, cefepime, cefluprenam, cefoselis, cefozopran, cefpirome, cefquinome, flomoxef. ceftobiprole, azithromycin, clarithromycin, dirithromycin, erythromycin, roxithromycin, aztreonam, pencillin and penicillin derivatives, actinomycin, bacitracin, colistin, polymyxin B, cinoxacin, flumequine, nalidixic acid, oxolinic acid, piromidic acide, pipemidic acid, rosoxacin, ciprofloxacin, enoxacin, fleroxacin, lomefloxacin, nadifloxacin, norfloxacin, ofloxacin, pefloxacin, rufloxacin, balofloxacin, gatifloxacin, grepafloxacin, levofloxacin, moxifloxacin, pazufloxacin, sparfloxacin, temafloxacin, tosufloxacin, clinafloxacin, garenoxacin, gemifloxacin, stifloxacin, trovalfloxacin,

prulifloxacin, acetazolamide, benzolamide, bumetanide, celecoxib, chlorthalidone, clopamide, dichlorphenamide, dorzolamide, ethoxyzolamide, furosemide, hydrochlorothiazide, indapamide, mafendide, mefruside, metolazone, probenecid, sulfacetamide, sulfadimethoxine, sulfadoxine, sulfanilamides, sulfamethoxazole, sulfasalazine, sultiame, sumatriptan, xipamide, tetracycline, chlortetracycline, oxytetracycline, doxycycline, lymecycline, meclocycline, methacycline, minocycline, rolitetracycline, methicillin, nafcillin, oxacilin, cloxacillin, vancomycin, teicoplanin, clindamycin, co-trimoxazole, and any combination thereof in amounts that are effective to additively or synergistically enhance the therapeutic effect of the bacteriophage and/or polypeptide of the invention for a given infection.

[0119] Local anesthetics that may be used in pharmaceutical compositions of the present invention include tetracaine, tetracaine hydrochloride, lidocain, lidocaine hydrochloride, dimethisoquin hydrochloride, dibucaine, dibucaine hydrochloride, butambenpicrate, and pramoxine hydrochloride. An exemplary concentration of local anesthetic is about 0.025% to about 5% by weight of the total composition.

[0120] Corticosteroids that may be useful in combination with the polypeptides, bacteriophage, and/or pharmaceutical compositions of the invention include betamethasone, dipropionate, fluocinolone, actinide, betamethasone valerate, triamcinolone actinide, clobetasol propionate, desoximetasone, diflorasone diacetate, amcinonide, flurandrenolide, hydrocortisone valerate, hydrocortisone butyrate, and desonide. An exemplary concentration of corticosteroid is about 0.01% to about 1% by weight of the total composition.

[0121] In certain embodiments, a formulation comprising a bacteriophage and/or polypeptide of the invention further comprises SM buffer (0.05 M Tris-HCl (pH 7.4-7.5); 0.1 M NaCl; 10 mM $MgSO_4$). In other embodiments, the formulation further comprises SM buffer and 10 mM $MgCl_2$. In still other embodiments, the formulation further comprises SM buffer and about 20% or about 30% ethanol.

[0122] Pharmaceutical compositions comprising a bacteriophage and/or polypeptide of the present invention can be formulated in a unit dose or multi-dose formulation. Suitable formulations can be selected from the group consisting of ointments, solutions, suspensions or emulsions, extracts, powders, granules, sprays, lozenges, tablets, or capsules; and additionally may include a dispersing agent or a stabilizing agent.

[0123] The pharmaceutical compositions of the invention can be administered by inhalation, in the form of a suppository or pessary, topically (*e.g.*, in the form of a lotion, solution, cream, ointment or dusting powder), epi- or transdermally (*e.g.*, by use of a skin patch), orally (*e.g.*, as a tablet, which may contain excipients such as starch or lactose), as a capsule, ovule, elixirs, solutions, or suspensions (each optionally containing flavoring, coloring agents and/or excipients), or they can be injected parenterally (*e.g.*, intravenously, intramuscularly or subcutaneously). For parenteral administration, the compositions may be used in the form of a sterile aqueous solution which may contain other substances, for example enough salts or monosaccharides to make the solution isotonic with blood. For buccal or sublingual administration the compositions may be administered in the form of tablets or lozenges which can be formulated in a conventional manner. In a preferred embodiment, a bacteriophage and/or polypeptide of the present invention is administered topically, either as a single agent, or in combination with other antibiotic treatments, as described herein or known in the art.

[0124] A bacteriophage and/or polypeptide of the present invention may also be dermally or transdermally administered. For topical application to the skin, the bacteriophage and/or polypeptides of the present invention may be combined with one or a combination of carriers, which can include but are not limited to, an aqueous liquid, an alcohol base liquid, a water soluble gel, a lotion, an ointment, a nonaqueous liquid base, a mineral oil base, a blend of mineral oil and petrolatum, lanolin, liposomes, proteins carriers such as serum albumin or gelatin, powdered cellulose carmel, and combinations thereof. A topical mode of delivery may include a smear, a spray, a bandage, a time-release patch, a liquid-absorbed wipe, and combinations thereof. The bacteriophage and/or polypeptide of the invention may be applied to a patch, wipe, bandage, etc., either directly or in a carrier(s). The patches, wipes, bandages, etc., may be damp or dry, wherein the phage and/or polypeptide (*e.g.*, a lysin) is in a lyophilized form on the patch. The carriers of topical compositions may comprise semi-solid and gel-like vehicles that include a polymer thickener, water, preservatives, active surfactants, or emulsifiers, antioxidants, sun screens, and a solvent or mixed solvent system. U.S. Patent No. 5,863,560 discloses a number of different carrier combinations that can aid in the exposure of skin to a medicament, and its contents are incorporated herein. The carrier may or may not involve a controlled-release formulation, *e.g.*, as disclosed in US 2008/0260697, the contents of which are incorporated herein by reference. The carrier may or may not involve phage adsorbed on a

matrix, *e.g.*, as described in any one of US 2008/0038322, US 2008/0138311, US 2009/0130196, EP 1 812 025, EP 1 817 043, and EP 1 833 497, the contents of which are incorporated herein by reference. In some embodiments, the carrier may or may not involve a viscous formulation, *e.g.*, a gel, *e.g.*, as disclosed in US 2009/0191254, the contents of which are incorporated herein by reference.

[0125] For intranasal or administration by inhalation, the bacteriophage and/or polypeptide of the invention is conveniently delivered in the form of a dry powder inhaler or an aerosol spray presentation from a pressurized container, pump, spray, or nebuliser with the use of a suitable propellant, *e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A.TM.) or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA.TM.), carbon dioxide, or other suitable gas. In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurized container, pump, spray, or nebuliser may contain a solution or suspension of the active compound, *e.g.*, using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, *e.g.*, sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of the bacteriophage and/or polypeptide of the invention and a suitable powder base such as lactose or starch.

[0126] For administration in the form of a suppository or pessary, the therapeutic compositions may be applied topically in the form of a gel, hydrogel, lotion, solution, cream, ointment, or dusting powder. Compositions of the invention may also be administered by the ocular route. For ophthalmic use, the compositions of the invention can be formulated as micronized suspensions in isotonic, pH adjusted, sterile saline, or, preferably, as solutions in isotonic, pH adjusted, sterile saline, optionally in combination with a preservative such as a benzylalkonium chloride. Alternatively, they may be formulated in an ointment such as petrolatum.

[0127] Dosages and desired drug concentrations of the pharmaceutical compositions of the present invention may vary depending on the particular use. The determination of the appropriate dosage or route of administration is within the skill of an ordinary physician. Animal experiments can provide reliable guidance for the determination of effective doses in human therapy. Interspecies scaling of effective doses can be performed by one of ordinary skill in the art following the principles described by Mordenti, J. and Chappell, W. "The use of interspecies

scaling in toxicokinetics" in Toxicokinetics and New Drug Development, Yacobi et al., Eds., Pergamon Press, New York 1989, pp42-96.

6.3 THERAPEUTIC USE

[0128] The bacteriophage and polypeptides of the present invention have activity against a plurality of strains of *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*, *e.g.*, as described in Tables 1-7, in the Examples below. Therefore, the compositions of the present invention may find use in methods of preventing and/or treating infections associated with *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus* in both humans and animals. In other embodiments, the compositions of the present invention may be used to treat infection associated with related species or strains of these bacteria, including, but not limited to *S. epidermidis, S. auricularis, S. capitis, S. haemolyticus, S. hominis, S. saprophyticus, S. simulans, S. xylosis, Micrococcus luteus, Bacilus subtilis, B. pumilus, E. hirae.*

[0129] In specific embodiments, the subject receiving a pharmaceutical composition of the invention is a mammal (*e.g.*, bovine, ovine, caprine, equid, primate (*e.g.*, human), rodent, lagomorph or avian (*e.g.*, chicken, duck, goose)). In the context of the present invention, "treatment" refers to therapeutic treatment, wherein the object is to eliminate, lessen, decrease the severity of, ameliorate, slow the progression of or prevent the symptoms or underlying cause (*e.g.*, bacterial infection) associated with the pathological condition or disorder. "Treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to eliminate, lessen, decrease the severity of, slow the progression of or delay or prevent the symptoms or underlying cause (*e.g.*, bacterial infection) associated with the pathological condition or disorder. It is also contemplated that a bacteriophage and/or polypeptide of the invention acts as a prophylactic or preventative measure, preventing the onset of infection caused by one or more bacteria.

[0130] *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and *S. aureus* are responsible for many severe opportunistic infections, particularly in individuals with compromised immune systems. The pharmaceutical compositions of the present invention are contemplated for treating any infection associated with *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus,* or associated with other species or strains of bacteria, including, but not limited to, infections of the skin (including but not limited to skin ulcers, carbuncles, bed sores, and diabetic

foot ulcers), infections in and around wounds, post-operative infections, infections associated with catheters and surgical drains, and infections of the blood.

[0131] Diabetic foot ulcer is one of the major complications of diabetes mellitus, occurring in about 15% of all diabetic patients and resulting in about 85% of all lower leg amputations. (Brem, et al., J. Clinical Invest., 2007, 117(5):1219–1222). Diabetes mellitus impedes the normal steps of the wound healing process. Non-healing chronic diabetic ulcers are often treated with extracellular matrix replacement therapy, advanced moist wound therapy, bio-engineered tissue or skin substitutes, growth factors, debridement, arterial revascularization, and/or negative pressure wound therapy. (Blume et.al, *Diabetes Care*, 2008, 31: 631-636). The ulcers may become infected with opportunistic bacteria, which further exacerbate the condition. Accordingly, foot ulcers in diabetes also often require antibiotics, *e.g.*, against *Staphylococcus*, as well as other anaerobic bacteria, such as *Klebsiella pneumonia, Escherchia coli*, and/or *Pseudomonas aeruginosa*.

[0132] One or more compositions of the present invention find use in the treatment of diabetic foot ulcer. For example, an isolated phage or polypeptide of the invention can be used for the treatment of infections associated with diabetic foot ulcer, in a subject in need thereof. In particular embobdiments, the composition used for treating diabetic foot ulcer is a topical composition, formulated for topical administration, *e.g.*, a composition for direct application to an ulcer, wound, lesion, and/or sore associated with diabetic foot ulcer.

[0133] In certain embodiments, the composition for use with respect to diabetic foot ulcer comprises isolated F44/10, having a genome comprising the nucleic acid sequence of SEQ ID NO:560 and exhibiting antibacterial activity against one or more strains of *Staphylococcus aureus*. In some embodiments, a composition is used comprising a polypeptide isolated from bacteriophage F44/10, or a fragment, variant, or derivative thereof, which polypeptide, fragment, variant, or derivative exhibits antibacterial activity against one or more species or strains of *S. aureus*. In certain such embodiments, the polypeptide, or fragment, variant, or derivative thereof, is a lysin, a CHAP domain, or a tail protein, exhibiting antibacterial activity against one or more species or strains of *S. aureus*. In certain embodiments, the composition for use with respect to diabetic foot ulcer comprises isolated F125/10, having a genome comprising the nucleic acid sequence of SEQ ID NO:1074 and exhibiting antibacterial activity against one or more strains of *Staphylococcus aureus*. In some embodiments, a composition is used comprising

a polypeptide isolated from bacteriophage F125/10, or a fragment, variant, or derivative thereof, which polypeptide, fragment, variant, or derivative exhibits antibacterial activity against one or more species or strains of *S. aureus.* In certain such embodiments, the polypeptide, or fragment, variant, or derivative thereof, is a lysin, a CHAP domain, or a tail protein, exhibiting antibacterial activity against one or more species or strains of *S. aureus.*

[0134] In certain embodiments, a composition comprising a phage cocktail is used, *e.g.*, where the phage cocktail comprises at least one phage exhibiting antibacterial activity against one or more strains of *Staphylococcus aureus* and at least one phage exhibiting antibacterial activity against a different bacteria. In particular embodiments, the phage cocktail comprises F44/10 and/or F125/10 in combination with at least one other phage selected from F391/08, F394/08, F488/08, F510/08, F387/08, F170/08, F168/08, F770/05, and F1245/05. In particularly preferred embodiments, the phage cocktail comprises F44/10 and/or F125/10 in combination with one, two, three or more other phages selected from F391/08, F387/08, F488/08, F510/08 and/or F770/05.

[0135] In certain embodiments, the composition for use with respect to diabetic foot ulcer comprises isolated F391/08 and/or F387/08, having a genome comprising the nucleic acid sequence of SEQ ID NO:1 or SEQ ID NO: 781, respectively, and exhibiting antibacterial activity against one or more strains of *Klebsiella pneumoniae.* In some embodiments, a composition is used comprising a polypeptide isolated from bacteriophage F391/08 and/or F387/08, or a fragment, variant, or derivative thereof, which polypeptide, fragment, variant, or derivative exhibits antibacterial activity against one or more species or strains of *K. pneumoniae.* In certain such embodiments, the polypeptide, or fragment, variant, or derivative thereof, is a lysin, a CHAP domain, or a tail protein, exhibiting antibacterial activity against one or more species or strains of *K. pneumoniae.* In certain embodiments, a composition comprising a phage cocktail is used, *e.g.*, where the phage cocktail comprises at least one phage exhibiting antibacterial activity against one or more strains of *Klebsiella pneumoniae* and at least one phage exhibiting antibacterial activity against a different bacteria. In particular embodiments, the phage cocktail comprises F391/08 and/or F387/08 in combination with at least one other phage selected from F394/08, F488/08, F510/08, F44/10, F170/08, F168/08, F770/05, F1245/05, and F125/10. In particularly preferred embodiments, the phage cocktail comprises F391/08 and/or F387/08 in

combination with one, two, three or more other phages selected from F44/10, F488/08, F510/08 and/or F770/05.

[0136] In certain embodiments, the composition for use with respect to diabetic foot ulcer comprises isolated F488/08, having a genome comprising the nucleic acid sequence of SEQ ID NO:3 and exhibiting antibacterial activity against one or more strains of *Escherichia coli.* In some embodiments, a composition is used comprising a polypeptide isolated from bacteriophage F488/08, or a fragment, variant, or derivative thereof, which polypeptide, fragment, variant, or derivative exhibits antibacterial activity against one or more species or strains of *E. coli.* In certain such embodiments, the polypeptide, or fragment, variant, or derivative thereof, is a lysin, a CHAP domain, or a tail protein, exhibiting antibacterial activity against one or more species or strains of *Escherichia coli.* In certain embodiments, a composition comprising a phage cocktail is used, *e.g.*, where the phage cocktail comprises at least one phage exhibiting antibacterial activity against one or more strains of *Escherichia coli* and at least one phage exhibiting antibacterial activity against a different bacteria. In particular embodiments, the phage cocktail comprises F488/08 in combination with at least one other phage selected from F394/08, F510/08, F44/10, F170/08, F168/08, F770/05, F1245/05, F391/08 F387/08, and F125/10. In particularly preferred embodiments, the phage cocktail comprises F488/08 in combination with one, two, three or more other phages selected from F391/08, F387/08, F44/10, F125/10, F510/08 and/or F770/05.

[0137] In certain embodiments, the composition for use with respect to diabetic foot ulcer comprises isolated F510/08 and/or F770/05, having a genome comprising the nucleic acid sequence of SEQ ID NO:4 or as disclosed in WO 2010/090542, respectively, and exhibiting antibacterial activity against one or more strains of *Pseudomonas aeruginosa.* In some embodiments, a composition is used comprising a polypeptide isolated from bacteriophage F510/08 and/or F770/05, or a fragment, variant, or derivative thereof, which polypeptide, fragment, variant, or derivative exhibits antibacterial activity against one or more species or strains of *P. aeruginosa.* In certain such embodiments, the polypeptide, or fragment, variant, or derivative thereof, is a lysin, a CHAP domain, or a tail protein, exhibiting antibacterial activity against one or more species or strains of *Pseudomonas aeruginosa.* In certain embodiments, a composition comprising a phage cocktail is used, *e.g.*, where the phage cocktail comprises at least one phage exhibiting antibacterial activity against one or more strains of *Pseudomonas*

aeruginosa and at least one phage exhibiting antibacterial activity against a different bacteria. In particular embodiments, the phage cocktail comprises F510/08 and/or F770/05 in combination with at least one other phage selected from F394/08, F488/08, F44/10, F170/08, F168/08, F1245/05, F391/08, F387/08, and F125/10. In particularly preferred embodiments, the phage cocktail comprises F510/08 and/or F770/05 in combination with one, two, three or more other phages selected from F44/10, F488/08, F391/08, and/or F387/08.

[0138] *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and *S. aureus* are also associated with infections that involve organ systems that have a high fluid content, and it is contemplated that the bacteriophage and/or polypeptides of the invention have therapeutic use in the prevention and treatment of these infections. For example, the pharmaceutical compositions of the invention may be used for the prevention or treatment of infections of the respiratory tract, of the cerebrospinal fluid, of peritoneal fluid, and of the urinary tract. The compositions of the invention may also be used to prevent and/or treat nosocomial pneumonia, infections associated with continuous ambulatory peritoneal dialysis (CAPD), catheter-associated bacterimia, and nosocomial meningitis.

[0139] In a preferred embodiment, a bacteriophage and/or polypeptide of the invention is used prophylactically in hospital setting, particularly to prevent infections associated with wounds, ulcers, and openings in the skin, *e.g.*, due to catheterization and any other medical procedures or devices.

[0140] In certain embodiments, a bacteriophage and/or polypeptide of the invention is used as a single agent for treating or preventing infections associated with *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*, and/or other bacterial species. In other embodiments of the invention, a bacteriophage and/or polypeptide of the invention is used in combination with other agents, including other bacteriophage (for example, that target a different species or strain of bacteria), or with antibiotics that target the same or different kinds of bacteria, including bacteria selected from any gram-positive bacteria, any gram-negative bacteria, and any other groups of bacteria that is not classified as gram-positive or gram-negative. The compositions of the invention may also be used in combination with any other means of treating bacterial infection known to one of skill in the art.

[0141] Also contemplated by the invention are methods of preventing and methods of treating an infection caused by bacteria including, but not limited to, *K. pneumoniae*, *A. baumannii*, *E. coli*,

P. aeruginosa, and/or *S. aureus* comprising administering to a mammal in need thereof a composition comprising a lysin comprising or consisting of the amino acid sequence of SEQ ID NO: 20, SEQ ID NO: 80, SEQ ID NO: 192, SEQ ID NO: 282, SEQ ID NO: 547, SEQ ID NO: 556, SEQ ID NO: 557, SEQ ID NO: 598, SEQ ID NO: 1216, SEQ ID NO: 1261, or a fragment, variant, or derivative thereof, wherein the fragment, variant, or derivative exhibits antibacterial or antimicrobial activity against the species of bacteria from which the parent bacteriophage was isolated. In a specific example in accordance with this embodiment, the invention provides methods of preventing or treating an infection caused by a bacteria including, but not limited to, *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*, comprising administering to a mammal in need thereof a composition comprising an isolated CHAP domain of a lysin, or a fragment, variant, or derivative thereof that exhibits at least one biologic activity of the CHAP domain from which it was isolated (*e.g.*, lytic cell killing).

[0142] In certain embodiments, the invention provides methods of preventing and/or treating an infection caused by bacteria including, but not limited to, *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*, comprising administering to a mammal in need thereof a composition comprising a tail protein comprising or consisting of the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 629, or SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, SEQ ID NOs: 1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1217, SEQ ID NO: 1250, SEQ ID NO: 1266, or a fragment, variant, or derivative thereof, wherein the fragment, variant, or derivative exhibits a biologic activity associated with the bacteriophage from with it was derived.

[0143] In still other embodiments, the invention provides methods of preventing and/or treating an infection caused by bacteria including, but not limited to, *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*, comprising administering to a mammal in need thereof a composition comprising bacteriophage having a genome comprising or consisting of the nucleic acid sequence of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO: 4, SEQ ID NO: 560, SEQ ID NO: 781, and/or SEQ ID NO: 1074. Combinations of the lysins (or fragments,

variants, or derivatives thereof as described above) and tail proteins (or fragments, variants, or derivatives thereof as described above), optionally with one or more bacteriophage of the invention or with other treatments, such as antibiotics, are also contemplated, as well as methods of treating and/or methods of preventing a bacterial infection using one or more of the combinations herein described.

[0144] As used herein, the term "in combination" refers to the use of more than one prophylactic and/or therapeutic agent. The use of the term "in combination" does not restrict the order in which prophylactic and/or therapeutic agents are administered to a subject with a disease or disorder. A first prophylactic or therapeutic agent can be administered prior to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (*e.g.*, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) the administration of a second prophylactic or therapeutic agent (different from the first prophylactic or therapeutic agent) to a subject with a disease or disorder.

6.4 DISINFECTANT AND ANTI-INFECTIVE USE

[0145] Bacterial pathogens most often infect at a mucous membrane site (*e.g.*, upper and lower respiratory, intestinal, urogenital, ocular, and the like). The mucous membranes themselves are often the reservoir, sometimes the only reservoir, for many pathogenic bacteria found in the environment (*e.g.*, pneumococci, staphylococci, and streptococci). There are very few anti-infectives that are designed to control the carrier state of pathogenic bacteria. However, studies have shown that by reducing or eliminating this reservoir in environments such as hospitals and nursing homes, the incidence of infections by these bacteria will be markedly reduced. The prevention of nosocomical infections involves routine and repeated cleaning of affected surfaces.

[0146] The bacteriophage and/or polypeptides of the present invention may be used in anti-infective compositions for controlling the growth of bacteria (*e.g.*, Gram-positive bacteria (*e.g.*, *S. aureus*), Gram-negative bacteria (*e.g.*, *K. pneumoniae*, *A. baumannii*, *E. coli*, and *P. aeruginosa*), or bacteria not classified as either Gram-positive or Gram-negative), in order to prevent or reduce the incidence of nocosomial infections. In addition to use in compositions for application to mucous membranes, a bacteriophage and/or polypeptide of the present

incorporation may also be incorporated into formulations such as gels, creams, ointments, or sprays for controlling or preventing colonization of bacteria on body surfaces (*e.g.*, skin and mucus membranes) (*e.g.*, for sterilization of surgical fields or of the hands and exposed skin of healthcare workers and/or patients) and other solid surfaces (*e.g.*, appliances, countertops, and, in particular, hospital equipment).

6.5 USE IN NANOTECHNOLOGY

[0147] The bacteriophage and/or polypeptides of the present invention also may be used in nanotechnology, *e.g.*, in the development of nanoscale devices. The combination of nanotechnology and molecular biology has led to a new generation of nanoscale-based devices, such as nanoscale conductors. Biological systems function based on the structure of macromolecules, mainly proteins and nucleic acids, which are structurally organized at the nanoscale. Accordingly, biological macromolecules may find use in nanoscale applications. In particular, proteins with highly organized structures can be used in the development of nanoscale devices.

[0148] In some embodiments, a polypeptide of the invention comprising or consisting of a tail protein (*e.g.*, tail component, tail fiber protein, adsorption associated tail protein, tail length tape measure protein, baseplate wedge subunit), or fragment, variant, or derivative thereof, isolated from a bacteriophage having a genome comprising or consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO:4, SEQ ID NO: 560, SEQ ID NO: 781, or SEQ ID NO: 1074 (*e.g.*, bacteriophage F391/08, F394/08, F488/08, F510/08, F44/10, F387/08, or F125/10, respectively), may be used in nanotechnological applications. For example, tail proteins from tail fibers of the phage may have highly organized structures and may find use in nanoscale conductors. Such conductors may be used, *e.g.*, to deposit gold and/or other ions.

[0149] In specific embodiments, the polypeptide of the invention used in nanotechonology is an isolated tail protein comprising or consisting of the amino acid sequence SEQ ID NO: 15, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 629, SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, SEQ ID NOs:

1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1217, SEQ ID NO: 1250, or SEQ ID NO: 1266. In other embodiments, the polypeptide of the invention comprises a fragment, variant or derivative of SEQ ID NO: 15, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 629, SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, SEQ ID NOs: 1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1217, SEQ ID NO: 1250, or SEQ ID NO: 1266, wherein said fragment, variant or derivative possesses a highly-organized structure. Such polypeptides find use, *e.g.*, in nanoscale conductors, as described above.

6.6 DIAGNOSTIC METHODS

[0150] The present invention also encompasses diagnostic methods for determining the causative agent in a bacterial infection. In certain embodiments, the diagnosis of the causative agent in a presentation of bacterial infection is performed by (i) culturing tissue, blood, or fluid samples of a patient according to standard techniques; (ii) contacting the culture with one or more bacteriophage and/or polypeptides of the invention; and (iii) monitoring cell growth and/or evidence of lysis after said contacting. Because the activity of bacteriophage and/or their isolated products (*e.g.*, polypeptides, or biologically active fragments, variants, or derivatives thereof) tends to be species or strain specific, susceptibility, or lack of susceptibility, to one or more bacteriophage and/or polypeptides of the invention may be indicative of the species or strain of infective bacteria. For example, decreased growth of test cultures after contacting with a bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 1 or 781, or with an isolated polypeptide thereof or derived therefrom, may be indicative of the test sample comprising *K. pneumoniae*. Similarly, a bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 2, or an isolated polypeptide product thereof or derived therefrom, may be used to identify infection by *A. baumannii*; a bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 3, or an isolated polypeptide product thereof or derived therefrom, may be used to identify infection by *E. coli*; while that having a genome comprising or consisting of the nucleic acid

sequence SEQ ID NO: 4, or an isolated polypeptide product thereof or derived therefrom, may be used to identify infection by *P. aeruginosa*; and that having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 560 or 1074, or an isolated polypeptide product thereof or derived therefrom, may be used to identify infection by *S. aureus*.

[0151] Additionally, in some embodiments, bacteriophage and/or polypeptides of the present invention may be used in biosensors in the scope of diagnostics. As used herein, "biosensor" refers an analytical device for the detection of an analyte that combines a biological component with a physicochemical detector component. In particular, proteins involved in the recognition of bacterial receptors can be used in the development of diagnostic biosensors.

[0152] In some embodiments, a polypeptide of the invention comprising or consisting of a tail protein (*e.g.*, tail component, tail fiber protein, adsorption associated tail protein, tail length tape measure protein, baseplate wedge subunit), or fragment, variant, or derivative thereof, isolated from a bacteriophage having a genome comprising or consisting of SEQ ID NO: 1, SEQ ID NO: 2, SEQ ID NO: 3, SEQ ID NO:4, SEQ ID NO: 560, SEQ ID NO: 781, SEQ ID NO: 1074 (*e.g.*, bacteriophage F391/08, F394/08, F488/08, F510/08, F44/10, F387/08, or F125/10 respectively), may be used in biosensor applications. For example, a phage tail protein may specifically recognize one or more bacterial species and/or strains, and thus may find use in biosensor diagnostics. The detection of a certain baterial species and/or strain, by one or more bacteriophage and/or polypeptides of the invention, can indicate the species or strain of infective bacteria.

[0153] In specific embodiments, the polypeptide of the invention used in biosensor applications is an isolated tail protein comprising or consisting of the amino acid sequence SEQ ID NO: 15, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 629, SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, SEQ ID NOs: 1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1217, SEQ ID NO: 1250, or SEQ ID NO: 1266. In other embodiments, the polypeptide of the invention comprises a fragment, variant or derivative of SEQ ID NO: 15, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35,

SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 190, SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NOs: 433-437, SEQ ID NOs: 489-496, SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 549, SEQ ID NO: 551, SEQ ID NO: 629, SEQ ID NO: 686, SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, SEQ ID NOs: 1053-1060, SEQ ID NO: 1077, SEQ ID NO: 1217, SEQ ID NO: 1250, or SEQ ID NO: 1266, wherein said fragment, variant or derivative is capable of speceifically recognizing a bacteria, *e.g.*, a specific species and/or one or more specific strains of the bacteria. Such polypeptides find use, *e.g.*, in biosensors for detecting specific bacteria and/or diagnosing certain infections, as described above.

[0154] Generally, the phage tail protein for use in a biosensor will detect its host bacteria, or one or more specific species and/or specific strains of the host bacteria. Accordingly, in certain embodiments, the invention encompasses a tail protein corresponding to the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, or SEQ ID NOs: 32-35, or a variant, fragment or derivative thereof, which recognizes and can detect one or more strains of *K. pneumoniae*. Such detection may be indicative of a *K. pneumoniae* infection. In certain embodiments, the invention encompasses a tail protein corresponding to the amino acid sequence of SEQ ID NO: 180, SEQ ID NO: 183, SEQ ID NO: 185, or SEQ ID NO: 190, or a variant, fragment or derivative thereof, which recognizes and can detect one or more strains of *A. baumannii*. Such detection may be indicative of an *A. baumannii* infection. In certain embodiments, the invention encompasses a tail protein corresponding to the amino acid sequence of SEQ ID NO: 231, SEQ ID NO: 232, SEQ ID NO:235, SEQ ID NOs: 239-245, SEQ ID NO: 248, SEQ ID NO: 249, SEQ ID NO: 252, SEQ ID NO: 254, SEQ ID NOs: 433-437, SEQ ID NOs: 489-495, or SEQ ID NO: 496, or a variant, fragment or derivative thereof, which recognizes and can detect one or more strains of *E. coli*. Such detection may be indicative of an *E. coli* infection

[0155] In certain embodiments, the invention encompasses a tail protein corresponding to the amino acid sequence of SEQ ID NO: 544, SEQ ID NO: 545, SEQ ID NO: 549, or SEQ ID NO: 551, or a variant, fragment or derivative thereof, which recognizes and can detect one or more strains of *P. aeruginosa*. Such detection may be indicative of a *P. aeruginosa* infection. In certain embodiments, the invention encompasses a tail protein corresponding to the amino acid

sequence of SEQ ID NO: 629, SEQ ID NO: 686, SEQ ID NO: 1077, SEQ ID NO: 1217, SEQ ID NO: 1250, or SEQ ID NO: 1266, or a variant, fragment or derivative thereof, which recognizes and can detect one or more strains of *S. aureus*. Such detection may be indicative of an *S. aureus* infection. In certain embodiments, the invention encompasses a tail protein corresponding to the amino acid sequence of SEQ ID NO: 789, SEQ ID NOs: 796-800, SEQ ID NO: 806, SEQ ID NO: 854, SEQ ID NOs: 999-1004, or SEQ ID NOs: 1053-1060, or a variant, fragment or derivative thereof, which recognizes and can detect one or more strains of *K. pneumoniae.* Such detection may be indicative of a *K. pneumoniae* infection.

[0156] In some embodiments, the invention encompasses the use of more than one tail protein, *e.g.*, a combination of two of more of the tail proteins provided above, in a biosensor for detecting more than one bacterial species and/or strains. The biosensor also may comprise additional proteins and/or other agents for detecting the same or different bacteria.

6.7 AMINO ACID VARIANTS

[0157] Amino acid sequence variants of the polypeptides of the invention can be created. In some embodiments, they may be substitutional, insertional and/or deletion variants. Deletion variants lack one or more residues of the native protein which typically are not essential for function (*e.g.*, antimicrobial or antibacterial activity). Insertional mutants typically involve the addition of material at a non-terminal point in the polypeptide. Substitutional variants typically involve the exchange of one amino acid for another at one or more sites within the polypeptide, and may be designed to modulate one or more properties of the polypeptide, such as stability against proteolytic cleavage, preferably without the loss (or substantial loss) of other functions or properties. Substitutions of this kind preferably are conservative, that is, one amino acid is replaced with one of similar shape and charge. Conservative substitutions are well known in the art and include, for example, the changes of: alanine to serine; arginine to lysine; asparagine to glutamine or histidine; aspartate to glutamate; cysteine to serine; glutamine to asparagine; glutamate to aspartate; glycine to proline; histidine to asparagine or glutamine; isoleucine to leucine or valine; leucine to valine or isoleucine; lysine to arginine; methionine to leucine or isoleucine; phenylalanine to tyrosine, leucine or methionine; serine to threonine; threonine to serine; tryptophan to tyrosine; tyrosine to tryptophan or phenylalanine; and valine to isoleucine or leucine.

[0158] Once general areas of the gene are identified as encoding a particular antibacterial activity, *e.g.*, as a lysin as described herein, point mutagenesis may be employed to identify with greater particularity which amino acid residues are important in the antibacterial activity. Thus, one of skill in the art will be able to generate, for example, single base changes in the DNA strand to result in an altered codon and/or a missense mutation that preserves desired function.

[0159] Preferably, mutation of the amino acids of a protein creates an equivalent, or even an improved, second-generation molecule. For example, certain amino acids may be substituted for other amino acids in a protein structure without detectable or substantial loss of function (*e.g.*, antibacterial or antimicrobial activity). In making such changes, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biological function on a protein is generally understood in the art. It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, interaction with a peptidoglycan within the outer coat of a gram-positive bacteria. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics; for example: isoleucine(+4.5); valine(+4.2); leucine(+3.8) ; phenylalanine(+2.8); cysteine/cystine(+2.5); methionine(+1.9); alanine(+1.8); glycine(-0.4); threonine(-0.7); serine(-0.8); tryptophan 0.9); tyrosine(-1.3); proline(-1.6); histidine(-3.2); glutamate(-3.5); glutamine(-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5). It is also understood in the art that the substitution of like amino acids can be made effectively on the basis of hydrophilicity. Like hydrophobicity, values of hydrophilicity have been assigned to each amino acid: arginine (+3.0); lysine (+3.0); aspartate (+3.0 $\pm$ 1); glutamate (+3.0 $\pm$ 1); serine (+0.3); asparagine (+0.2); glutamine (+0.2); glycine (0); threonine (-0.4); proline (-0.5 $\pm$ 1); alanine (-0.5); histidine (-0.5); cysteine (-1.0); methionine (-1.3); valine (-1.5); leucine (-1.8); isoleucine (-1.8); tyrosine (-2.3); phenylalanine (-2.5) and tryptophan (-3.4). Equivalent molecules may be obtained by substitution of one amino acid for another where their hydropathic and/or their hydrophilicity indices are within $\pm$ 2, preferably $\pm$ 1, or most preferably $\pm$ 5 of each other.

[0160] In certain embodiments, the invention encompasses isolated peptides that comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid modifications (*e.g.*, insertion, substitution, deletion, etc.) relative to an amino acid sequence disclosed herein. In preferred embodiments, the

mutation(s) are made such that biological activity of the particular polypeptide is retained or substantially retained. For example, the present invention encompasses polypeptides isolated from bacteriophage F387/08, F391/08, F394/08, F488/08, F510/068, F44/10, and/or F125/10, which are mutated to comprise 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 or more amino acid modifications relative to an amino acid sequence listed herein, and which exhibit antibacterial activity against one or more species or strains of Gram-positive or Gram-negative bacterium, *e.g.*, against *K. pneumoniae*, *A. baumannii*, *E. coli*, *P. aeruginosa*, and/or *S. aureus*. In specific embodiments, the polypeptides of the invention derived from F387/08 or F391/08 exhibit antibacterial or antimicrobial activity, *e.g.*, lytic killing activity, against at least *K. pneumoniae*; those derived from F394/08 against at least *A. baumannii*; those derived from F488/08 against at least *E. coli*; those derived from F510/08 against at least. *P. aeruginosa*, and those derived from F44/10 or F125/10 against at least. *S. aureus*.

6.8 POLYNUCLEOTIDES ENCODING POLYPEPTIDES OF THE INVENTION

[0161] The invention provides polynucleotides comprising a nucleotide sequence encoding a polypeptide of the invention. The invention also encompasses polynucleotides that hybridize under high stringency, intermediate, or lower stringency hybridization conditions, *e.g.*, as defined *supra*, to polynucleotides that encode a polypeptide of the invention and that encode modified polypeptides that have antibiotic and/or other biological activity.

[0162] The polynucleotides may be obtained, and the nucleotide sequence of the polynucleotides determined, by any method known in the art. For example, a polynucleotide encoding a polypeptide of the invention may be generated from nucleic acid from a suitable source (*e.g.*, bacteriophage F387/08, F391/08, F394/08, F488/08, F510/08, F44/10, and/or F125/10). Nucleotide sequences may be isolated from phage genomes by routine methods known in the art (see, *e.g.*, Carlson, "Working with bacteriophage: common techniques and methodological approaches," *In,* Kutter and Sulakvelidze (Eds) Bacteriophage: Biology and Applications, 5th ed. CRC Press (2005); incorporated herein by reference in its entirety). If a source containing a nucleic acid encoding a particular polypeptide is not available, but the amino acid sequence of the polypeptide of the invention is known, a nucleic acid encoding the polypeptide may be

chemically synthesized and cloned into replicable cloning vectors using any method well known in the art.

[0163] Once the nucleotide sequence of the polypeptide of the invention is determined, the nucleotide sequence of the polypeptide may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook et al., 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.*, eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY, which are both incorporated by reference herein in their entireties), to generate polypeptides having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

[0164] In some embodiments, a nucleotide sequence encoding one or more ORFs of Figures 2, 4, 6, 8, 10, 12, and 14 are provided. In some embodiments, a nucleotide sequence encoding a variant, fragment or derivative of one or more ORFs of Figures 2, 4, 6, 8, 10, 12, and 14 are provided, where the variant, fragment or derivative exhibits antibacterial or antimicrobial activity (*e.g.*, lytic killing activity) against one or more strains of *K. pneumoniae*, for example, against the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 1 or SEQ ID NO: 781; and/or one against or more strains of *A. baumannii*, for example, against the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 2; and/or against one or more strains of *E. coli*, for example, against the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 3; against one or more strains of *P. aeruginosa*, for example, against the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 4; and/or against one or more strains of *S. aureus*, for example, against the bacteriophage having a genome comprising or consisting of the nucleic acid sequence SEQ ID NO: 560 or SEQ ID NO: 1074.

6.9 RECOMBINANT EXPRESSION OF MOLECULES OF THE INVENTION

[0165] Once a nucleic acid sequence encoding a molecule of the invention (*e.g.*, a polypeptide) has been obtained, the vector for the production of the molecules may be produced by recombinant DNA technology using techniques well known in the art. Methods which are well known to those skilled in the art can be used to construct expression vectors containing the

coding sequences for the molecules of the invention and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. (*See*, for example, the techniques described in Sambrook *et al.*, 1990, Molecular Cloning, A Laboratory Manual, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY and Ausubel *et al.* eds., 1998, Current Protocols in Molecular Biology, John Wiley & Sons, NY).

[0166] The present invention provides expression vectors encoding the polypeptides of the invention. An expression vector comprising the nucleotide sequence of a molecule identified by the methods of the invention can be transferred to a host cell by conventional techniques (*e.g.*, electroporation, liposomal transfection, and calcium phosphate precipitation) and the transfected cells are then cultured by conventional techniques to produce the molecules of the invention. In preferred embodiments, the host cell is other than the species of the parent bacteria from which the bacteriophage comprising the sequence was derived. In specific embodiments, the expression of the molecules of the invention is regulated by a constitutive, an inducible or a tissue, specific promoter. In specific embodiments the expression vector is pQE-30 (Qiagen) or pET-29(a) (Novagen).

[0167] The host cells used to express the molecules identified by the methods of the invention may be either bacterial cells (preferably non susceptible to the bacteriophage protein or variant, derivative, or fragment thereof of the invention). A variety of host-expression vector systems may be utilized to express the molecules identified by the methods of the invention. Such host-expression systems represent vehicles by which the coding sequences of the molecules of the invention may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express the molecules of the invention *in situ*. These include, but are not limited to, microorganisms such as bacteria that are not susceptible to the bacteriophage protein or variant, derivative, or fragment thereof of the invention (*e.g.*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA, or cosmid DNA expression vectors containing coding sequences for the molecules identified by the methods of the invention; yeast (*e.g.*, *Saccharomyces Pichia*) transformed with recombinant yeast expression vectors containing sequences encoding the molecules identified by the methods of the invention; insect cell systems infected with recombinant virus expression vectors (*e.g.*, baculovirus) containing the sequences encoding the

molecules identified by the methods of the invention; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus (CaMV) and tobacco mosaic virus (TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing sequences encoding the molecules identified by the methods of the invention; or mammalian cell systems (*e.g.*, COS, CHO, BHK, 293, 293T, 3T3 cells, lymphotic cells (*see* U.S. 5,807,715), Per C.6 cells (human retinal cells developed by Crucell) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter) containing sequences encoding the molecules identified by the methods of the invention.

[0168] In bacterial systems not susceptible to the bacteriophage protein or variant, derivative, or fragment thereof of the invention, a number of expression vectors may be advantageously selected depending upon the use intended for the molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of pharmaceutical compositions of a polypeptide, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther *et al.*, 1983, *EMBO J.* 2:1791), in which the protein sequence may be ligated individually into the vector in frame with the *lac Z* coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, 1985, *Nucleic Acids Res.* 13:3101-3109; Van Heeke & Schuster, 1989, *J. Biol. Chem.* 24:5503-5509); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free gluta-thione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0169] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes. The virus preferably grows in *Spodoptera frugiperda* cells. The polypeptide coding sequence may be cloned individually into non-essential regions (*e.g.,* the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (*e.g.*, the polyhedrin promoter).

[0170] Once a molecule of the invention (*i.e.*, polypeptides) has been recombinantly expressed, it may be purified by any method known in the art for purification of polypeptides, for example, by chromatography (*e.g.*, ion exchange, affinity, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of polypeptides or antibodies.

[0171] Certain modifications and improvements will occur to those skilled in the art upon a reading of the foregoing description. It should be understood that all such modifications and improvements have been deleted herein for the sake of conciseness and readability but are properly within the scope of the following claims.

7. EXAMPLES

[0172] It is understood that the following examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggestive to persons skilled in the art and are to be included within the spirit and purview of this application and the scope of the appended claims. All publications, patents, and patent applications cited herein are hereby incorporated by reference in their entirety for all purposes.

[0173] Unless otherwise indicated, the bacteriophage of the invention were isolated, processed and analyzed according to the following methods.

7.1.1 PURIFICATION OF PHAGE

[0174] Stock preparations of bacteriophage isolated from clinical samples were prepared according to protocols described in Carlson, "Working with bacteriophage: common techniques and methodological approaches," *In,* Kutter and Sulakvelidze (Eds) Bacteriophage: Biology and Applications, 5th ed. CRC Press (2005) ("Carlson," incorporated herein by reference in its entirety).

[0175] The bacteriophage stock preparations were concentrated by precipitation with PEG according to the protocol described in Carlson and Yamamoto et al., 2004, *PNAS* 101:6415-6420. Briefly, the stock preparation was incubated in 1 M NaCl for one hour at 4°C with agitation. Next, PEG 8000 (AppliChem, Cheshire, MA) was gradually added to a final concentration of 10% (w/v). The composition was then incubated overnight at 4°C. After the incubation period, the composition was centrifuged at 10000 x g for 30 minutes at 4°C. The sediment was then re-suspended in SM buffer (0.05 M Tris-HCL at pH 7.4, 0.1 M NaCl, 10 mM

$MgSO_4$) with gelatin at 1% w/v and centrifuged again at 1000 rpm at 4°C for 10 minutes. The supernatant containing the suspended phage was saved for further purification. The supernatant was purified using a CsCl gradient according to the methods in Carlson.

[0176] CsCl was removed from the purified and concentrated phage stock by dialysis. A dialysis membrane, Cellu.Sep H1 High Grade Regenerated Cellulose Tubular Membrane (Cellu.Sep, River Street, USA), was prepared according to the manufacturers' instructions. The dialysis involved a first incubation of 30 minutes in 100 mM Tris-HCl and 3 M NaCl (pH 7.4) at 4°C. This was followed by a second incubation of 30 minutes in 100 mM Tris-HCl and 0.3 M NaCl (pH 7.4) at 4°C. After dialysis, the suspended phage was removed from the interior of the dialysis bag and stored at 4°C.

7.1.2 EXTRACTION OF PHAGE DNA

[0177] To 5 ml of the purified and concentrated bacteriophage samples was added 20 mM EDTA at pH 8.0, SDS at 0.5% (p/v) and Proteinase K at a final concentration of 40 µg/ml. The mixture was incubated at 56°C for one hour. Successive extractions in phenol:chloroform:alcohol at a proportions of 25:24:1 were performed until the interface between the aqueous and organic phases was clear. The aqueous phase was then treated with an equal volume of chloroform and centrifuged at 13,0000 x g for 10 minutes at 4°C. The aqueous phase was once again removed, and the DNA was precipitated by adding two volumes of absolute ethanol and incubating for thirty minutes at 20°C. The samples were then centrifuged at 11,000 x g for 30 minutes at 4°C. The pellet was washed with 70% ethanol at room temperature and resuspended in 50 µl of ultra-pure water (Gibco, California). DNA concentration was determined by measuring the absorbance at 260 nm in a ND-1000 Spectrophotometer. Integrity of the isolated phage DNA was analyzed by electrophoresis on a 1% agarose gel.

7.1.3 ANALYSIS OF PHAGE GENOMES

[0178] Sequencing of the bacteriophage genome allowed identification of potential open reading frames (ORFs) within the genome. The putative ORFs of the bacteriophage were used to search the NCBI nucleotide collection database for homologous DNA sequences using the BLASTN program (see, *e.g.*, Zhang et al., 2000, *J. Comput. Biol.* 7:203-214).

7.2 EXAMPLE 1: Bacteriophage F391/08

[0179] Comparison of the putative ORFs of the bacteriophage F391/08 genome with the sequences in the NCBI nucleotide database revealed that only small portions of the genome (≤ 11% genome coverage) exhibited homology with known sequences. A schematic organization of the F391/08 genome is provided in FIG. 1. F391/08 ORFs, their encoded amino acid sequences, and known homologous proteins are provided in FIG. 2. Prediction of *orfs* was performed by integrating the results obtained with GeneMark.hmm and MetaGeneAnnotator programs (Besemer, J. and Borodovsky, M. 1999. Nucleic Acids Res., 27: 3911-3920; Noguchi, H. et al., 2008. DNA Res., 15: 387-396). Protein homology searches were carried out with BLASTP program (Alschul, S.F. et al., 1997. Nucleic Acids Res., 25: 3389-33402) using the NCBI non-redundant protein sequences database. Protein conserved domains were predicted using NCBI specialized BLAST (Marchler-Bauer, A. et al., 2007. Nucleic Acids Res. 35: 237-240). *orfs* whose products presented homology with the same protein(s) are indicated with the same number with the addition of a lowercase letter, in FIG. 2. Identification of putative transfer RNA genes (tRNA) was carried out using the tRNAscan-SE progam (Lowe, T.M. et al., 1997. Nucleic Acids Res., 25: 955-964).

[0180] Table 1 below provides the results of spot tests that assessed the host range and activity of the bacteriophage F391/08 against 100 *Klebsiella* sp. strains (86 *K. pneumoniae* strains; 12 *K. oxytoca* strains; and 2 *Klebsiella* sp. strains) isolated from clinical samples. Each spot contained 5 µl of bacteriophage suspension with the indicated titers, prepared from a CsCl purified lysate. Sensitivity of each strain to the phage was evaluated based on a relative scale ranging from turbid (+) to clear (++++) lysis halos. Spots originating from isolated phage plaques are indicated as (pfu) and resistance to phage infection is indicated as (-). The percentage of strains displaying a particular sensitivity phenotype is indicated also.

Table 1

Phage	Titer (pfu/ml)	Phage sensitivity (%) of *Klebsiella* sp. strains (n=100)						Total of infected strains (%)
		++++	+++	++	+	pfu	-	
F391/08	$2.72x10^{10}$	3	26	8	11	0	52	**48**
	$2.72x10^{8}$	1	5	6	1	0	87	**13**
	$2.72x10^{6}$	0	1	3	1	1	94	**6**
	$2.72x10^{4}$	0	0	0	0	3	97	**3**

7.3 EXAMPLE 2: Bacteriophage F394/08

[0181] Comparison of the putative ORFs of the bacteriophage F394/08 genome with the sequences in the NCBI nucleotide database revealed no significant homologies with known sequences, other than that observed for small portions of the genome (≤ 1% genome coverage). A schematic organization of the F394/08 genome is provided in FIG. 3. F394/08 ORFs, their encoded amino acid sequences, and known homologous proteins are provided in FIG. 4. Prediction of *orfs* was performed by integrating the results obtained with GeneMark.hmm and MetaGeneAnnotator programs (Besemer, J. and Borodovsky, M. 1999. Nucleic Acids Res., 27: 3911-3920; Noguchi, H. et al., 2008. DNA Res., 15: 387-396). Protein homology searches were carried out with BLASTP program (Alschul, S.F. et al., 1997. Nucleic Acids Res., 25: 3389-33402) using the NCBI non-redundant protein sequences database. Protein conserved domains were predicted using NCBI specialized BLAST (Marchler-Bauer, A. et al., 2007. Nucleic Acids Res. 35: 237-240). *orfs* whose products presented homology with the same protein(s) are indicated with the same number with the addition of a lowercase letter, in FIG. 4. Identification of putative transfer RNA genes (tRNA) was carried out using the tRNAscan-SE progam (Lowe, T.M. et al., 1997. Nucleic Acids Res., 25: 955-964).

[0182] Table 2 below provides the results of spot tests that assessed the host range and activity of the bacteriophage F394/08 against 100 *Acinetobacter* sp. strains (93 *A. baumannii* strains; 6 *A.calcoaceticus* strains; and 1 *A. lwoffi* strain) isolated from clinical samples. Each spot contained 5 µl of bacteriophage suspension with the indicated titers, prepared from a CsCl purified lysate. Sensitivity of each strain to the phage was evaluated based on a relative scale ranging from turbid (+) to clear (++++) lysis halos. Spots originating from isolated phage plaques are indicated as (pfu) and resistance to phage infection is indicated as (-). The percentage of strains displaying a particular sensitivity phenotype is indicated also.

Table 2

Phage	Titer (pfu/ml)	Phage sensitivity (%) of *Acinetobacter* sp. strains (n=100)						Total of infected strains (%)
		++++	+++	++	+	pfu	-	
F394/08	$2.2x10^9$	67	3	1	0	0	29	**71**
	$2.2x10^8$	66	1	2	1	0	30	**70**
	$2.2x10^6$	9	38	8	0	0	45	**55**

	$2.2x10^4$	0	7	1	0	32	60	**40**

7.4 EXAMPLE 3: Bacteriophage F488/08

[0183] Comparison of the putative ORFs of the bacteriophage F488/08 genome with the sequences in the NCBI nucleotide database revealed that approximately 94% of phage F488/08 DNA is highly similar to that of Enterobaceria phage RB14, with individual ORF identities ranging from 70 to 100%. A schematic organization of the F488/08 genome is provided in FIG. 5. F4884/08 ORFs, their encoded amino acid sequences, and known homologous proteins are provided in FIG. 6. Prediction of *orfs* was performed by integrating the results obtained with GeneMark.hmm and MetaGeneAnnotator programs (Besemer, J. and Borodovsky, M. 1999. Nucleic Acids Res., 27: 3911-3920; Noguchi, H. et al., 2008. DNA Res., 15: 387-396). Protein homology searches were carried out with BLASTP program (Alschul, S.F. et al., 1997. Nucleic Acids Res., 25: 3389-33402) using the NCBI non-redundant protein sequences database. Protein conserved domains were predicted using NCBI specialized BLAST (Marchler-Bauer, A. et al., 2007. Nucleic Acids Res. 35: 237-240). *orfs* whose products presented homology with the same protein(s) are indicated with the same number with the addition of a lowercase letter, in FIG. 4. Identification of putative transfer RNA genes (tRNA) was carried out using the tRNAscan-SE progam (Lowe, T.M. et al., 1997. Nucleic Acids Res., 25: 955-964).

[0184] Table 3 below provides the results of spot tests that assessed the host range and activity of the bacteriophage F488/08 against 100 *Escherichia coli* (ECO) strains isolated from clinical samples. Each spot contained 5 µl of bacteriophage suspension with the indicated titers, prepared from a lysate purified by ion exchange chromatography. Sensitivity of each strain to the phage was evaluated based on a relative scale ranging from turbid (+) to clear (++++) lysis halos. Spots originating from isolated phage plaques are indicated as (pfu) and resistance to phage infection is indicated as (-). The percentage of strains displaying a particular sensitivity phenotype is indicated also.

Table 3

Phage	Titer (pfu/ml)	Phage sensitivity (%) of ECO strains (n=100)						Total of infected strains (%)
		++++	+++	++	+	pfu	-	
F488/08	$1x10^{10}$	0	60	1	2	0	37	**63**
	$1x10^8$	0	42	15	0	0	43	**57**

	$1x10^6$	0	8	10	7	0	75	**25**
	$1x10^4$	0	0	0	0	10	90	**10**

7.5 EXAMPLE 4: Bacteriophage F510/08

[0185] Comparison of the putative ORFs of the bacteriophage F510/08 genome with the sequences in the NCBI nucleotide database revealed that approximately 95% of phage F510/08 DNA is highly similar to that of Pseudomonas phage LUZ19, with individual ORF identities ranging from 89 to 97%. A schematic organization of the F510/08 genome is provided in FIG. 7. F510/08 ORFs, their encoded amino acid sequences, and known homologous proteins are provided in FIG. 8. Prediction of *orfs* was performed by integrating the results obtained with GeneMark.hmm and MetaGeneAnnotator programs (Besemer, J. and Borodovsky, M. 1999. Nucleic Acids Res., 27: 3911-3920; Noguchi, H. et al., 2008. DNA Res., 15: 387-396). Protein homology searches were carried out with BLASTP program (Alschul, S.F. et al., 1997. Nucleic Acids Res., 25: 3389-33402) using the NCBI non-redundant protein sequences database. Protein conserved domains were predicted using NCBI specialized BLAST (Marchler-Bauer, A. et al., 2007. Nucleic Acids Res. 35: 237-240). *orfs* whose products presented homology with the same protein(s) are indicated with the same number with the addition of a lowercase letter, in FIG. 8. Identification of putative transfer RNA genes (tRNA) was carried out using the tRNAscan-SE progam (Lowe, T.M. et al., 1997. Nucleic Acids Res., 25: 955-964).

[0186] Table 4 below provides the results of spot tests that assessed the host range and activity of the bacteriophage F510/08 against 100 Pseudomonas aeruginosa (PSA) strains isolated from clinical samples. Each spot contained 5 µl of bacteriophage suspension with the indicated titers, prepared from a CsCl purified lysate. Sensitivity of each strain to the phage was evaluated based on a relative scale ranging from turbid (+) to clear (++++) lysis halos. Spots originating from isolated phage plaques are indicated as (pfu) and resistance to phage infection is indicated as (-). The percentage of strains displaying a particular sensitivity phenotype is indicated also.

Table 4

Phage	Titer (pfu/ml)	Phage sensitivity (%) of PSA strains (n=100)						Total of infected strains (%)
		++++	+++	++	+	pfu	-	
F510/08	$5.7x10^{10}$	35	18	4	10	3	30	**70**

	$5.7x10^8$	18	25	6	9	4	38	**62**
	$5.7x10^6$	13	14	4	2	4	63	**37**
	$5.7x10^4$	10	12	0	1	10	67	**33**

7.6 EXAMPLE 5: Bacteriophage F44/10

[0187] Comparison of the putative ORFs of the bacteriophage F44/10 genome with the sequences in the NCBI nucleotide database revealed that approximately 81% of phage F44/10 DNA is highly similar to that of Staphylococcus phage K, with individual ORF identities ranging from 80 to 99%. A schematic organization of the F44/10 genome is provided in FIG. 9. F44/10 ORFs, their encoded amino acid sequences, and known homologous proteins are provided in FIG. 10. Prediction of *orfs* was performed by integrating the results obtained with GeneMark.hmm and MetaGeneAnnotator programs (Besemer, J. and Borodovsky, M. 1999. *Nucleic Acids Res.*, 27: 3911-3920; Noguchi, H. et al., 2008. *DNA Res.*, 15: 387-396). Protein homology searches were carried out with BLASTP program (Alschul, S.F. et al., 1997. *Nucleic Acids Res.*, 25: 3389-33402) using the NCBI non-redundant protein sequences database. Protein conserved domains were predicted using NCBI specialized BLAST (Marchler-Bauer, A. et al., 2007. *Nucleic Acids Res.* 35: 237-240). *orfs* whose products presented homology with the same protein(s) are indicated with the same number with the addition of a lowercase letter, in FIG. 10. As previously reported for Staphylococcus phage K, the putative polymerase gene (orf114a, orf114b) may contain an intron-like sequence. (O'Flaherty et al., 2004). Identification of putative transfer RNA genes (tRNA) was carried out using the tRNAscan-SE progam (Lowe, T.M. et al., 1997. *Nucleic Acids Res.*, 25: 955-964).

[0188] Table 5 below provides the results of spot tests that assessed the host range and activity of the bacteriophage F44/10 against 100 *Staphylococcus aureus* (STA) strains isolated from clinical samples. Each spot contained 5 µl of bacteriophage suspension with the indicated titers, prepared from a CsCl purified lysate. Sensitivity of each strain to the phage was evaluated based on a relative scale ranging from turbid (+) to clear (++++) lysis halos. Spots originating from isolated phage plaques are indicated as (pfu) and resistance to phage infection is indicated as (-). The percentage of strains displaying a particular sensitivity phenotype is indicated also.

Table 5

Phage	Titer (pfu/ml)	Phage sensitivity (%) of STA strains (n=100)						Total of infected strains (%)
		++++	+++	++	+	pfu	-	
F44110	$2.25x10^{11}$	53	35	10	1	1	0	**100**
	$2.25x10^{10}$	35	52	10	2	1	0	**100**
	$2.25x10^{8}$	19	51	5	5	1	19	**81**
	$2.25x10^{6}$	5	22	0	0	34	39	**61**
	$2.25x10^{4}$	0	0	0	0	42	58	**42**

7.7 EXAMPLE 6: Bacteriophage F387/08

[0189] Comparison of the putative ORFs of the bacteriophage F387/08 genome with the sequences in the NCBI nucleotide database revealed no signigicant homologies with known sequences other than small portions of the genome (≤ 12% genome coverage). A schematic organization of the F387/08 genome is provided in FIG. 11. Functionally assigned *orfs* are indicated on the right and in FIGs. 11B-C. DNA homology searches were carried out with BLASTN program (Zhang, Z. et al., 2000. J. Comput. Biol., 7: 203-214), using the NCBI nucleotide collection database.

[0190] F387/08 ORFs, their encoded amino acid sequences, and known homologous proteins are provided in FIG. 12. Prediction of *orfs* was performed by integrating the results obtained with GeneMark.hmm and MetaGeneAnnotator programs (Besemer, J. and Borodovsky, M. 1999. Nucleic Acids Res., 27: 3911-3920; Noguchi, H. et al., 2008. DNA Res., 15: 387-396). Protein homology searches were carried out with BLASTP program (Alschul, S.F. et al., 1997. Nucleic Acids Res., 25: 3389-33402) using the NCBI non-redundant protein sequences database. Protein conserved domains were predicted using NCBI specialized BLAST (Marchler-Bauer, A. et al., 2007. Nucleic Acids Res. 35: 237-240).

[0191] Table 6 below provides the results of spot tests that assessed the host range and activity of the bacteriophage F387/08 against 100 *Klebsiella* sp. strains (86 *K. pneumoniae* strains; 12 *K. oxytoca* strains; and 2 *Klebsiella* sp. strains) isolated from clinical samples. Each spot contained 5 µl of bacteriophage suspension with the indicated titers, prepared from a CsCl purified lysate. Sensitivity of each strain to the phage was evaluated based on a relative scale ranging from turbid (+) to clear (++++) lysis halos. Spots originating from isolated phage plaques are

indicated as (pfu) and resistance to phage infection is indicated as (-). The percentage of strains displaying a particular sensitivity phenotype is indicated also.

Table 6

Phage	Titer (pfu/ml)	Phage sensitivity (%) of KLE strains (n=100)						Total of infected strains (%)
		++++	+++	++	+	pfu	-	
F387/08	$1.0x10^{10}$	1	38	20	9	0	32	**68**
	$1.0x10^{8}$	0	10	14	9	0	67	**33**
	$1.0x10^{6}$	0	2	2	0	1	95	**5**
	$1.0x10^{4}$	0	0	0	1	2	97	**3**

7.8 EXAMPLE 7: Bacteriophage F125/10

[0192] Comparison of the putative ORFs of the bacteriophage F125/10 genome with the sequences in the NCBI nucleotide database revealed that approximately 87% of phage F125/10 DNA is highly similar to that of Staphylococcus phage A5W, with individual ORF identities ranging from 77 to 99%. A schematic organization of the F125/10 genome is provided in FIG. 13. F125/10 ORFs, their encoded amino acid sequences, and known homologous proteins are provided in FIG. 14. Prediction of *orfs* was performed by integrating the results obtained with GeneMark.hmm and MetaGeneAnnotator programs (Besemer, J. and Borodovsky, M. 1999. *Nucleic Acids Res.*, 27: 3911-3920; Noguchi, H. et al., 2008. *DNA Res.*, 15: 387-396). Protein homology searches were carried out with BLASTP program (Alschul, S.F. et al., 1997. *Nucleic Acids Res.*, 25: 3389-33402) using the NCBI non-redundant protein sequences database. Protein conserved domains were predicted using NCBI specialized BLAST (Marchler-Bauer, A. et al., 2007. *Nucleic Acids Res.* 35: 237-240). *orfs* whose products presented homology with the same protein(s) are indicated with the same number with the addition of a lowercase letter, in FIG. 14. As previously reported for Staphylococcus phage K (O'Flaherty et al., 2004, *J. of Bacteriology* 186(9):2862-2871), and phage Twort (Landthaler et al., 2002, *Nucleic Acids Research* 30(9):1935-1943), introns interrupting genes involved in DNA metabolism were found; and the putative terminase large subunit gene (orf153a, orf153b) may contain an intron-like sequence (orf154*).

[0193] Table 7 below provides the results of spot tests that assessed the host range and activity of the bacteriophage F125/10 against 98 *Staphylococcus aureus* (STA) strains isolated from clinical samples. Each spot contained 5 µl of bacteriophage suspension with the indicated titers, prepared from a CsCl purified lysate. Sensitivity of each strain to the phage was evaluated based on a relative scale ranging from turbid (+) to clear (++++) lysis halos. Phage dilutions originating from isolated phage plaques are indicated as (pfu) and resistance to phage infection is indicated as (-). The percentage of strains displaying a particular sensitivity phenotype is indicated also.

Table 7

Phage	Titer (pfu/ml)	Phage sensitivity (%) of STA strains (n=198)						Total of infected strains (%)
		++++	+++	++	+	pfu	-	
F125/10	$8.30x10^9$	49	27	12	10	0	0	**100**
	$8.30x10^8$	21	38	21	16	0	2	**98**
	$8.30x10^6$	4	29	27	15	0	23	**77**
	$8.30x10^4$	0	9	20	10	22	37	**62**

CLAIMS

1. An isolated bacteriophage having a genome which comprises at least 99% sequence identity to the nucleotide sequence of SEQ ID NO:1, and having antibacterial activity against *Klebsiella pneumoniae.*

2. A pharmaceutical composition comprising the bacteriophage of claim 1 and a pharmaceutically acceptable carrier.

3. The pharmaceutical composition of claim 2, further comprising one or more additional strains of bacteriophage effective against *Klebsiella pneumoniae.*

4. An isolated protein from bacteriophage F391/08 having a genome comprising at least 99% sequence identity to the nucleotide sequence of SEQ ID NO:1, and having antibacterial activity against *Klebsiella pneumoniae*, or a fragment of said protein having antibacterial activity against *Klebsiella pneumoniae*; wherein said protein is a lysin protein or a tail protein.

5. The isolated protein of claim 4, wherein said protein is a lysin protein having the amino acid sequence of SEQ ID NO: 20, SEQ ID NO: 80, or a fragment thereof, which fragment has antibacterial activity against *Klebsiella pneumoniae,* or wherein said protein is a tail protein having the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, or a fragment thereof, which fragment has antibacterial activity against *Klebsiella pneumoniae*.

6. An isolated first protein having at least 85% or at least 95% sequence identity to a second protein of the same size, wherein said first protein has antibacterial activity against *Klebsiella pneumoniae,*

and said second protein has the amino acid sequence of SEQ ID NO: 20, SEQ ID NO: 80, or a fragment thereof, or the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NOs: 32-35, or a fragment thereof.

7. The fragment of claim 4, 5 or 6, wherein said fragment comprises a cysteine histidine-dependent amidohydrolases/peptidase domain (CHAP domain) of the amino acid sequence of SEQ ID NO: 20 or SEQ ID NO: 80.

8. A pharmaceutical composition comprising the isolated protein or fragment of any one of claims 4, 5 or 6, or the fragment of claim 7 and a pharmaceutically acceptable carrier.

9. The pharmaceutical composition of claim 8, comprising the isolated protein having the amino acid sequence of SEQ ID NO: 15, SEQ ID NO: 20, SEQ ID NO: 26, SEQ ID NO: 27, SEQ ID NO: 30, SEQ ID NO: 32-35, SEQ ID NO: 80, or a fragment thereof having antibacterial activity against *Klebsiella pneumoniae.*

10. The pharmaceutical composition of claim 9, further comprising one or more bacteriophage or additional bacteriophage proteins effective against *Klebsiella pneumoniae.*

11. The pharmaceutical composition of claim 2 or 3, further comprising at least three additional phage selected from the group consisting of F394/08, having a genome comprising at least 99% sequence identity to the nucleotide sequence of SEQ ID NO:2; F488/08, having a genome comprising at least 99% sequence identity to the nucleotide sequence of SEQ ID NO:3; F510/08, having a genome comprising at least 99% sequence identity to the nucleotide sequence of SEQ ID NO:4; F44/10, having a genome comprising at least 99% sequence identity to

the nucleotide sequence of SEQ ID NO:560; F387/08, having a genome comprising at least 99% sequence identity to the nucleotide sequence of SEQ ID NO:781; F125/10, having a genome comprising at least 99% sequence identity to the nucleotide sequence of SEQ ID NO:1074; F170/08, having a genome comprising at least 99% sequence identity to the nucleotide sequence of SEQ ID NO:2 of WO 2010/090542; F168/08, having a genome comprising at least 99% sequence identity to the nucleotide sequence of SEQ ID NO:1 of WO 2010/090542; F770/05, having deposit number NCIMB 41864; and F1245/05, having deposit number NCIMB 41865; preferably at least three additional phage selected from the group consisting of F44/10, F387/08, F488/08, F510/08 and/or F770/05; preferably comprising F44/10; and/or wherein said composition is formulated for topical application.

12. An isolated nucleic acid comprising a nucleotide sequence encoding the protein or fragment of claim 4, 5, or 6 or fragment of claim 7.

13. Use of a therapeutically effective amount of the pharmaceutical composition of any of claims 2-3 or 8-11 in the manufacture of a medicament for treating or preventing a bacterial infection in a subject in need thereof; wherein said bacterial infection is an infection by *Klebsiella pneumoniae*

preferably wherein the infection is a nosocomial infection and/or wherein the subject is a mammal, preferably a human.

14. A method for diagnosing the causative agent of a bacterial infection comprising

(i) culturing a sample from a patient;

(ii) contacting the culture of step (i) with the bacteriophage from claim 1, the protein or fragment of claim 4, 5, or 6, or the fragment of claim 7; and

(iii) monitoring for evidence of growth or lysis of the culture

wherein evidence of lysis of the culture indicates that the culture comprises *Klebsiella pneumoniae*;

preferably wherein the sample is a sample of blood, urine, tissue biopsy, sputum, nasal mucosa, or swab collected from said patient.

15. A method for reducing or inhibiting colonization or growth of *Klebsiella pneumoniae* bacteria on a solid surface comprising contacting said surface with the bacteriophage of claim 1, the polypeptide or fragment of claim 4, 5, or 6, or the fragment of claim 7; preferably wherein said surface is the surface of a hospital apparatus or a piece of hospital equipment, preferably a surgical apparatus or piece of surgical equipment.

16. Use of the bacteriophage of claim 1, the polypeptide or fragment of claim 4, 5, or 6, or the fragment of claim 7 in the manufacture of a medicament for reducing or inhibiting colonization or growth of *Klebsiella pneumoniae* bacteria on a biological surface contacted therewith; preferably wherein said surface is the skin or a mucus membrane of a mammal, the skin or a mucus membrane of a human, or a mucus membrane of the respiratory tract of a mammal or human.

17. The use according to claim 13, wherein the infection is an infection of the skin.

18. The use according to claim 17, wherein the skin infection is associated with diabetic foot ulcer.

19. The use according to claim 17 or 18, wherein the composition is to be administered topically.

20. The use according to any one of claims 13 or 17-19, wherein the subject has a weakened immune system, preferably wherein the weakened immune system is a result of alcoholism or diabetes.

21. The use according to claim 13, wherein the infection is an infection associated with chronic pulmonary disease, nasal mucosa atrophy, or rhinoscleroma.

22. The use according to claim 21, wherein the pharmaceutical composition is to be administered by inhalation.

23. The use according to claim 22, wherein administration by inhalation uses a pump, a spray, or a nebulizer; and/or wherein the pharmaceutical composition for administration by inhalation comprises a dry powder or an aerosol spray.

24. The use according to claim 13, wherein the infection is an infection associated with enteric pathogenicity.

25. The use according to claim 24, wherein the pharmaceutical composition is to be administered orally or parenterally.

26. The use according to claim 13, wherein the pharmaceutical composition further comprises an antibiotic for treating said bacterial infection.