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)).