[0006] Among other things, the present disclosure provides compositions and methods for prevention and/or treatment of nosocomial infections in patient populations in need thereof.

[0007] Among other things, the present disclosure provides immunogenic compositions comprising, for example, (i) a backbone polymer comprising a polymer and one or more antigenic polysaccharides conjugated to the polymer; and (ii) one or more polypeptide antigens non-covalently complexed with the polymer or the antigenic polysaccharide.

[0007a] In one aspect, the present disclosure refers to an immunogenic composition comprising (i) a backbone polymer comprising a polymer and one or more antigenic polysaccharides conjugated to the polymer; and (ii) one or more polypeptide antigens non-covalently complexed with the polymer and/or the antigenic polysaccharide; wherein the polymer is a *Klebsiella spp*. K19 capsular polysaccharide.

[0008] In some examples, an immunogenic composition may comprise one or more antigenic polysaccharides conjugated to a polymer with a linker. In some examples, an immunogenic composition may comprise one or more antigenic polysaccharides conjugated to a polymer without a linker. In some examples, the one or more antigenic polysaccharides are derived from gram-negative bacteria and/or gram-positive bacteria. In some examples, the one or more antigenic polysaccharides are derived from *Klebsiella*, *Pseudomonas*, and/or *E. coli* antigenic polysaccharides.

[0009] In some examples, one or more of the opsonization potential, or immunogenicity of the one or more antigenic polysaccharides is increased relative to a predetermined level, as measured by ELISA and/or by an antibody functional assay. In some examples, one or more of the opsonization potential, or immunogenicity of the one or more antigenic polysaccharides is increased relative to a control composition, as measured by ELISA and/or by an antibody functional assay. In some examples, a control composition may comprise an antigenic polysaccharide present in the immunogenic composition and not comprising a backbone polymer present in the immunogenic composition. In some examples, a control composition may comprise an antigenic polypeptide present in the immunogenic composition and not comprising a backbone polymer present in the immunogenic composition. In some examples, a control composition may comprise an antigenic polypeptide present in the immunogenic composition and/or an antigenic polysaccharide present in the immunogenic composition, and not comprising a polymer present in the immunogenic composition. In some examples, a control composition may comprise an antigenic polysaccharide present in the vaccine composition and not comprising a backbone polymer present in the vaccine composition. In some

having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (*P. aeruginosa* PcrV) or an immunogenic fragment thereof.

[00037a] In another aspect, the present disclosure refers to a fusion protein comprising: (i) a biotin- binding protein, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (P. aeruginosa flagellin subtype B D2 domain lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least ~~80~~95% of the amino acid residues found in SEQ ID NO:10, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (P. aeruginosa PcrV) or an immunogenic fragment thereof comprising at least ~~80~~95% of the amino acid residues found in SEQ ID NO:8.

[00038] In some examples, at least one complementary affinity-molecule pair is or comprises a fusion protein comprising (i) a biotin-binding protein or biotin-binding domain thereof, (ii) a polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:7 (*P. aeruginosa* FliC subtype B flagellin) or an immunogenic fragment thereof, and (iii) a polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:2 or SEQ ID NO:3 (*K. pneumoniae* MrkA) or an immunogenic fragment thereof.

[00038a] In another aspect, the present disclosure refers to a fusion protein comprising: (i) a biotin- binding protein, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (P. aeruginosa FlaB flagellin D2 domain (FlaBD2) lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least ~~80~~95% of the amino acid residues found in SEQ ID NO:10, and (iii) a polypeptide comprising an amino acid sequence having at least 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:3 (K. pneumoniae MrkA) or an immunogenic fragment thereof comprising at least ~~80~~95% of the amino acid residues found in SEQ ID NO:3.

[00039] In some examples, at least one complementary affinity-molecule pair is or comprises a fusion protein comprising (i) a biotin-binding protein or biotin-binding domain thereof, (ii) a polypeptide comprising an amino acid sequence having at least 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (*P. aeruginosa* FliC subtype B flagellin D2 domain lacking the TLR5 binding motif) or an immunogenic fragment thereof, and (iii) a polypeptide comprising an amino acid sequence

cellular components include protein and/or nucleic acid, the method comprising steps of: contacting one or more cellular components with an oxidizing reagent to obtain a mixture, wherein the pH of the mixture is between about 3 and 5; separating the O-antigen polysaccharide from the cellular components; and recovering the OPS, wherein the OPS is substantially free of the other cellular components and contains a free aldehyde at its reducing terminal end. In some examples, the oxidizing reagent is sodium nitrite, the acid is acetic acid and wherein the reducing terminal end of the OPS is a 2,5-anhydromannose residue containing a free aldehyde.

[00049a] In another aspect, the present disclosure refers to a method of purifying an O-antigen polysaccharide from one or more cellular components of *Klebsiella*, wherein the cellular components include protein and/or nucleic acid, the method comprising steps of: contacting one or more cellular components with an oxidizing reagent and an acid to obtain a mixture, wherein the pH of the mixture is between ~~about~~ 3 and 5, and wherein the oxidizing agent is sodium nitrite and the acid is acetic acid; separating the O-antigen polysaccharide from the cellular components; and recovering the OPS, wherein the OPS is substantially free of the other cellular components and contains a 2,5-anhydromannose residue containing a free aldehyde at its reducing terminal end.

[00050] In another example, the present disclosure refers to a method of purifying an O-antigen polysaccharide from one or more cellular components of gram-negative bacteria, wherein the cellular components include protein and/or nucleic acid, the method comprising: contacting one or more cellular components with an acidic reagent to obtain a mixture wherein the pH of the mixture is between about 3 and 5, heating the mixture to between about 80°C to about 100°C; separating the O-antigen polysaccharide from the cellular components; and recovering the OPS, wherein the OPS is substantially free of the other cellular components and contains a ketone at its reducing terminal end.

[00051] In another example, the present disclosure refers to a method of making a backbone polymer comprising at least one non-biotinylated O-antigen polysaccharide, wherein the O-antigen polysaccharide comprises a primary amino group, and at least one biotinylated polysaccharide, the method comprising: chemically linking the O-antigen polysaccharide primary amino group by mixing the O-antigen polysaccharide with a partially oxidized polysaccharide containing aldehyde groups and a biotin containing primary amine to obtain a mixture; and reductively aminating the mixture with a reducing agent to produce a BP-1 polymer backbone, wherein the polysaccharide is biotinylated and the chemically linked O-antigen polysaccharide is not biotinylated. Some examples provide for a BP-1 polymer backbone made by the methods disclosed herein.

[00052] In another aspect, the present disclosure refers to a method of making a backbone polymer comprising at least one non-biotinylated O-antigen polysaccharide, wherein the O-antigen polysaccharide comprises a primary amino group, and at least one biotinylated polysaccharide, the method comprising: chemically linking the O-antigen polysaccharide primary amino group by mixing the O-antigen polysaccharide with a polysaccharide biotinylated and oxidized obtained by first treating the carboxylate groups of the polysaccharide with 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide N-

Claims

We claim:

1. An immunogenic composition comprising (i) a backbone polymer comprising a polymer and one or more antigenic polysaccharides conjugated to the polymer; and (ii) one or more polypeptide antigens non-covalently complexed with the polymer and/or the antigenic polysaccharide; wherein the polymer is a *Klebsiella spp*. K19 capsular polysaccharide.

2. The immunogenic composition of claim 1, wherein the one or more polypeptide antigens are non-covalently complexed with the polymer and/or one or more antigenic polysaccharides by formation of at least one complementary affinity-molecule pair comprising (i) a first affinity molecule associated with the polymer and/or one or more antigenic polysaccharides; and (ii) a complementary affinity molecule associated with one or more polypeptide antigens.

3. The immunogenic composition of claim 2, wherein the complementary affinity-molecule pair is selected from the group consisting of: biotin/biotin-binding protein, antibody/antigen, enzyme/substrate, receptor/ligand, metal/metal-binding protein, carbohydrate/carbohydrate binding protein, lipid/lipid-binding protein, and His tag/His tag-binding molecule.

4. The immunogenic composition of claim 2 or 3, wherein the first affinity molecule is cross-linked or covalently bonded to the polymer and/or the one or more antigenic polysaccharides.

5. The immunogenic composition of any one of claims 2-4, wherein the one or more polypeptide antigens comprise one or more *P. aeruginosa* proteins or variants comprising at least 95% identity to the one or more *P. aeruginosa* proteins, and wherein the complementary affinity molecule comprises a biotin-binding polypeptide.

6. The immunogenic composition of any one of claims 2-5, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising a biotin-binding polypeptide and a polypeptide comprising an amino acid sequence having at least 95%, 96%,

97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (FlaB flagellin D2 domain lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:10 or a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:11 (FlaA1 flagellin D2 domain lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:11 or a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:12 (FlaA2 flagellin D2 domain lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:12.

7. The immunogenic composition of any one of claims 2-6, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising a biotin-binding polypeptide and a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (FlaB flagellin D2 domain (FlaBD2) lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:10.

8. The immunogenic composition of any one of claims 2-7, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising a biotin-binding polypeptide and a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (*P. aeruginosa* type III secretion system (TTSS) PcrV) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:8.

9. The immunogenic composition of any one of claims 2-8, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:7 (*P. aeruginosa* flagellin subtype B) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:7, and (iii) a polypeptide comprising an amino acid sequence

having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (*P. aeruginosa* PcrV) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:8.

10. The immunogenic composition of any one of claims 2-9, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (*P. aeruginosa* flagellin subtype B D2 domain (FlaBD2) lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:10, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (*P. aeruginosa* PcrV) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:8.

11. The immunogenic composition of any one of claims 2-10, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:7 (*P. aeruginosa* subtype B flagellin) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:7, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:2 or SEQ ID NO:3 (*K. pneumoniae* MrkA) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:2 or SEQ ID NO:3.

12. The immunogenic composition of any one of claims 2-11, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (*P. aeruginosa* subtype B flagellin D2 domain (FlaBD2) lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:10, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or

100% identity to the sequence of SEQ ID NO:2 or SEQ ID NO:3 (*K. pneumoniae* MrkA) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:2 or SEQ ID NO:3.

13. The immunogenic composition of any one of claims 7-12, wherein upon administration to a subject, the immunogenic composition elicits antibodies that recognize native MrkA on *K. pneumoniae* expressing MrkA.

145. The immunogenic composition of any one of the preceding claims, wherein the one or more antigenic polysaccharides comprise a polysaccharide of one or more of *Klebsiella*, *Pseudomonas*, and *E. coli*.

15. The immunogenic composition of any one of the preceding claims, wherein the one or more antigenic polysaccharides comprises an O-antigen polysaccharide (OPS).

166. The immunogenic composition of claim 14 or 155, wherein the one or more antigenic polysaccharides comprise a *K. pneumoniae* OPS of type O1, O2, O3, O5, or a combination thereof.

177. The immunogenic composition of claim 14 or 155, wherein the one or more antigenic polysaccharides comprise a *P. aeruginosa* OPS of type O1, O2, O3, O4, O5, O6, O10, O11, O12, or a combination thereof.

188. The immunogenic composition of any one of the preceding claims, wherein at least one polypeptide antigen comprises an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:2 (*K. pneumoniae* conserved Type III fimbrial protein MrkA) and/or an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:3 or an immunogenic fragment thereof comprising at least 80%95% of the amino acid residues found in SEQ ID NO: 2 or SEQ ID NO: 3.

199. The immunogenic composition of any one of the preceding claims, wherein at least one polypeptide antigen is a *P. aeruginosa* flagellin subtype A, *P. aeruginosa* flagellin subtype B, or an immunogenic fragment thereof comprising at least 80%95% of the amino acid residues found in *P. aeruginosa* flagellin subtype A or *P. aeruginosa* flagellin subtype B.

2010. The immunogenic composition of any one of the preceding claims, wherein at least one polypeptide antigen comprises an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (*P. aeruginosa* PcrV), or an immunogenic fragment thereof comprising at least 80%95% of the amino acid residues found in SEQ ID NO:8.

21. The immunogenic composition of any one of the preceding claims, wherein at least one of the polypeptide antigens comprises an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:7 (*P. aeruginosa* FlaB flagellin) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:7.

2211. An immunogenic composition ~~of any of the preceding claims~~ comprising

(a) a backbone polymer comprising

(i) a polymer comprising a *Klebsiella spp.* K19 capsular polysaccharide; and

(ii) one or more antigenic polysaccharides comprising a polysaccharide of *Klebsiella* or *Pseudomonas* conjugated to the *Klebsiella spp.* K19 capsular polysaccharide; and

(b) one or more polypeptide antigens non-covalently complexed with the polymer by formation of at least one complementary affinity-molecule pair comprising

(i) a first affinity molecule associated with the polymer; and

(ii) a complementary affinity molecule associated with the one or more polypeptide antigens.

23. The immunogenic composition of claim 22, wherein the one or more antigenic polysaccharides comprises an O-antigen polysaccharide (OPS).

2412. The immunogenic composition of claim 22 or 2311, wherein the one or more antigenic polysaccharides comprise a *K. pneumoniae* OPS of type O1, O2, O3, O5, or a combination thereof.

2513. The immunogenic composition of any one of claims 22-24claim 11 or claim 12, wherein the one or more antigenic polysaccharides comprise a *P. aeruginosa* OPS of type O1, O2, O3, O4, O5, O6, O10, O11, or a combination thereof.

2614. The immunogenic composition of any one of claims 22-2511-13, wherein the first affinity molecule is biotin or a derivative thereof and the complementary affinity molecule is a biotin-binding polypeptide.

2715. The immunogenic composition of claim 2614, wherein the biotin-binding polypeptide is rhizavidin.

2816. The immunogenic composition of any one of claims 22-2711-15, wherein the one or more polypeptide antigens is selected from *P. aeruginosa* flagellin subtype B D2 domain (FlaBD2) lacking the TLR5 binding motif, *P. aeruginosa* PcrV, *K. pneumoniae* MrkA, antigenic fragments thereof comprising at least 80%95% of the residues found in *P. aeruginosa* flagellin subtype B D2 domain (FlaBD2) lacking the TLR5 binding motif, *P. aeruginosa* PcrV, or *K. pneumoniae* MrkA, or a combination thereof.

2917. The immunogenic composition of any one of claims 22-2811-16, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (FlaB flagellin D2 domain lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 80%95% of the amino acid residues found in SEQ ID NO:10, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:2 or SEQ ID NO:3 (*K. pneumoniae* MrkA) or an immunogenic

fragment thereof comprising at least ~~80%~~95% of the amino acid residues found in SEQ ID NO:2 or SEQ ID NO:3.

30~~18~~. The immunogenic composition of ~~any one of claims 11-17~~claim 29, wherein the fusion protein comprises a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:24 (Rhavi-FlaB-Domain2-MrkA-His).

31~~19~~. The immunogenic composition of any one of claims 22-28~~11-18~~, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (*P. aeruginosa* flagellin subtype B D2 domain (FlaBD2) lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least ~~80%~~95% of the amino acid residues found in SEQ ID NO:10, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (*P. aeruginosa* PcrV) or an immunogenic fragment thereof comprising at least ~~80%~~95% of the amino acid residues found in SEQ ID NO:8.

32~~20~~. The immunogenic composition of ~~any one of claims 11-19~~claim 31, wherein the fusion protein comprises a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:26 (Rhavi-FlaB-Domain2-PcrV-His).

33. The immunogenic composition of any one of claims 22-32, wherein the one or more polypeptide antigens comprise one or more *P. aeruginosa* proteins or variants comprising at least 95% identity to the one or more *P. aeruginosa* proteins, and wherein the complementary affinity molecule comprises a biotin-binding polypeptide.

34. The immunogenic composition of any one claims 22-33, wherein at least one of the polypeptide antigens comprises an amino acid sequence having at least 95%, 96%, 97%, 98%,

99%, or 100% identity to the sequence of SEQ ID NO:7 (*P. aeruginosa* FlaB flagellin) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:7.

35. The immunogenic composition of any one of claims 22-34, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising a biotin-binding polypeptide and a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (FlaB flagellin D2 domain lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:10 or a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:11 (FlaA1 flagellin D2 domain lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:11 or a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:12 (FlaA2 flagellin D2 domain lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:12.

36. The immunogenic composition of any one of claims 22-35, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising a biotin-binding polypeptide and a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (FlaB flagellin D2 domain (FlaBD2) lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:10.

37. The immunogenic composition of any one of claims 22-36, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising a biotin-binding polypeptide and a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (*P. aeruginosa* type III

secretion system (TTSS) PcrV) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:8.

38. The immunogenic composition of any one of claims 22-37, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:7 (*P. aeruginosa* flagellin subtype B) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:7, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (*P. aeruginosa* PcrV) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:8.

39. The immunogenic composition of any one of claims 22-38, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (*P. aeruginosa* flagellin subtype B D2 domain (FlaBD2) lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:10, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (*P. aeruginosa* PcrV) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:8.

40. The immunogenic composition of any one of claims 22-39, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:7 (*P. aeruginosa* subtype B flagellin) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:7, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:2 or

SEQ ID NO:3 (*K. pneumoniae* MrkA) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:2 or SEQ ID NO:3.

41. The immunogenic composition of any one of claims 22-40, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (*P. aeruginosa* subtype B flagellin D2 domain (FlaBD2) lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:10, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:2 or SEQ ID NO:3 (*K. pneumoniae* MrkA) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:2 or SEQ ID NO:3.

42. The immunogenic composition of any one of claims 36-41, wherein upon administration to a subject, the immunogenic composition elicits antibodies that recognize native MrkA on *K. pneumoniae* expressing MrkA.

43~~21~~. A vaccine composition comprising:

(a) a first immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a KP O1 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-MrkA fusion protein non-covalently complexed to the biotinylated backbone polymer;

(b) a second immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a KP O2 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-PcrV fusion protein non-covalently complexed to the biotinylated backbone polymer;

(c) a third immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a

KP O3 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-MrkA fusion protein non-covalently complexed to the biotinylated backbone polymer; and

(d) a fourth immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a KP O5 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-PcrV fusion protein non-covalently complexed to the biotinylated backbone polymer.

4422. A vaccine composition comprising:

(a) a first immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O1 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-PcrV fusion protein non-covalently complexed to the biotinylated backbone polymer;

(b) a second immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O2 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-PcrV fusion protein non-covalently complexed to the biotinylated backbone polymer;

(c) a third immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O3 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-PcrV fusion protein non-covalently complexed to the biotinylated backbone polymer;

(d) a fourth immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O4 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-PcrV fusion protein non-covalently complexed to the biotinylated backbone polymer;

(e) a fifth immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O5 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-MrkA fusion protein non-covalently complexed to the biotinylated backbone polymer;

(f) a sixth immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O6 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-MrkA fusion protein non-covalently complexed to the biotinylated backbone polymer;

(g) a seventh immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O10 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-MrkA fusion protein non-covalently complexed to the biotinylated backbone polymer; and

(h) an eighth immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O11 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-MrkA fusion protein non-covalently complexed to the biotinylated backbone polymer.

45~~23~~. A vaccine composition comprising:

(a) a first immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a KP O1 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-MrkA fusion protein non-covalently complexed to the biotinylated backbone polymer;

(b) a second immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a KP O2 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a

Rhavi-FlaBD2-PcrV fusion protein non-covalently complexed to the biotinylated backbone polymer;

(c) a third immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a KP O3 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-MrkA fusion protein non-covalently complexed to the biotinylated backbone polymer;

(d) a fourth immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a KP O5 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-PcrV fusion protein non-covalently complexed to the biotinylated backbone polymer;

(e) a fifth immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O1 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-PcrV fusion protein non-covalently complexed to the biotinylated backbone polymer;

(f) a sixth immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O2 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-PcrV fusion protein non-covalently complexed to the biotinylated backbone polymer;

(g) a seventh immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O3 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-PcrV fusion protein non-covalently complexed to the biotinylated backbone polymer;

(h) an eighth immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O4 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and

a Rhavi-FlaBD2-PcrV fusion protein non-covalently complexed to the biotinylated backbone polymer;

(i) a ninth immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O5 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-MrkA fusion protein non-covalently complexed to the biotinylated backbone polymer;

(j) a tenth immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O6 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-MrkA fusion protein non-covalently complexed to the biotinylated backbone polymer;

(k) an eleventh immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O10 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-MrkA fusion protein non-covalently complexed to the biotinylated backbone polymer; and

(l) a twelfth immunogenic composition comprising a backbone polymer comprising a polymer comprising a biotinylated *Klebsiella spp.* K19 capsular polysaccharide, a PA O11 OPS conjugated to the *Klebsiella spp.* K19 capsular polysaccharide, and a Rhavi-FlaBD2-MrkA fusion protein non-covalently complexed to the biotinylated backbone polymer.

46. The vaccine composition of any one of claims 43-45, wherein the Rhavi-FlaBD2-MrkA fusion protein and/or the Rhavi-FlaBD2-PcrV fusion protein comprises a biotin-binding polypeptide and a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (FlaB flagellin D2 domain (FlaBD2) lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:10.

47. The vaccine composition of any one of claims 43-45, wherein the Rhavi-FlaBD2-PcrV fusion protein comprises a biotin-binding polypeptide and a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (*P. aeruginosa* type III secretion system (TTSS) PcrV) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:8.

48. The vaccine composition of any one of claims 43-45, wherein Rhavi-FlaBD2-PcrV fusion protein comprises (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (*P. aeruginosa* flagellin subtype B D2 domain (FlaBD2) lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:10, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (*P. aeruginosa* PcrV) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:8.

49. The vaccine composition of any one of claims 43-45, wherein the Rhavi-FlaBD2-MrkA fusion protein (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (*P. aeruginosa* subtype B flagellin D2 domain (FlaBD2) lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:10, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:2 or SEQ ID NO:3 (*K. pneumoniae* MrkA) or an immunogenic fragment thereof comprising at least 95% of the amino acid residues found in SEQ ID NO:2 or SEQ ID NO:3.

50. The vaccine composition of any one of claims 43-45, wherein upon administration to a subject, the vaccine composition elicits antibodies that recognize native MrkA on *K. pneumoniae* expressing MrkA.

5124. The vaccine composition of any one of claims 43-4521-23, wherein the Rhavi-FlaBD2-MrkA fusion protein comprises a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:24 (Rhavi-FlaB-Domain2-MrkA-His).

5225. The vaccine composition of any one of claims 43-4521-23, wherein the Rhavi-FlaBD2-PcrV fusion protein comprises a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:26 (Rhavi-FlaB-Domain2-PcrV-His).

5326. The immunogenic composition or vaccine composition of any one of the preceding claims, wherein upon administration to a subject, the immunogenic composition elicits an immune response against gram-negative and/or gram-positive bacteria.

5427. The immunogenic composition or vaccine composition of claim 5326, wherein the immune response is an antibody or B cell response.

5528. The immunogenic composition or vaccine composition of claim 5326, wherein the immune response is a CD4+ T cell response, including Th1, Th2, or Th17 response, or a CD8+ T cell response, or CD4+/CD8+ T cell response.

5629. The immunogenic composition or vaccine composition of claim 5326, wherein the immune response is:

an antibody or B cell response; and

a T cell response.

5730. The immunogenic composition or vaccine composition of any one of claims 53-5626-29, wherein the gram-negative bacteria are selected from *Klebsiella, Pseudomonas, E. coli,* and a combination thereof.

31. The immunogenic composition or vaccine composition of any one of claims 2-30, wherein the one or more polypeptide antigens comprise one or more *P. aeruginosa* proteins or variants comprising at least 95% identity to the one or more *P. aeruginosa* proteins, and wherein the complementary affinity molecule comprises a biotin-binding polypeptide.

32. The immunogenic composition or vaccine composition of any one claims 2-31, wherein at least one of the polypeptide antigens comprises an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:7 (*P. aeruginosa* FlaB flagellin) or an immunogenic fragment thereof comprising at least 80% of the amino acid residues found in SEQ ID NO:7.

33. The immunogenic composition or vaccine composition of any one of claims 2-32, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising a biotin-binding polypeptide and a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (FlaB flagellin D2 domain lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 80% of the amino acid residues found in SEQ ID NO:10 or a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:11 (FlaA1 flagellin D2 domain lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 80% of the amino acid residues found in SEQ ID NO:11 or a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:12 (FlaA2 flagellin D2 domain lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 80% of the amino acid residues found in SEQ ID NO:12.

34. The immunogenic composition or vaccine composition of any one of claims 2-33, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising a biotin-binding polypeptide and a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (FlaB flagellin D2 domain (FlaBD2) lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 80% of the amino acid residues found in SEQ ID NO:10.

35. The immunogenic composition or vaccine composition of any one of claims 2-34, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising a biotin-binding polypeptide and a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (*P. aeruginosa* type III secretion system (TTSS) PcrV) or an immunogenic fragment thereof comprising at least 80% of the amino acid residues found in SEQ ID NO:8.

36. The immunogenic composition or vaccine composition of any one of claims 2-35, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:7 (*P. aeruginosa* flagellin subtype B) or an immunogenic fragment thereof comprising at least 80% of the amino acid residues found in SEQ ID NO:7, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (*P. aeruginosa* PcrV) or an immunogenic fragment thereof comprising at least 80% of the amino acid residues found in SEQ ID NO:8.

37. The immunogenic composition or vaccine composition of any one of claims 2-36, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (*P. aeruginosa* flagellin subtype B D2 domain (FlaBD2) lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 80% of the amino acid residues found in SEQ ID NO:10, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (*P. aeruginosa* PcrV) or an immunogenic fragment thereof comprising at least 80% of the amino acid residues found in SEQ ID NO:8.

38. The immunogenic composition or vaccine composition of any one of claims 2-37, wherein at least one complementary affinity-molecule pair comprises a fusion protein

~~comprising (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:7 (*P. aeruginosa* subtype B flagellin) or an immunogenic fragment thereof comprising at least 80% of the amino acid residues found in SEQ ID NO:7, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:2 or SEQ ID NO:3 (*K. pneumoniae* MrkA) or an immunogenic fragment thereof comprising at least 80% of the amino acid residues found in SEQ ID NO:2 or SEQ ID NO:3.~~

~~39. The immunogenic composition or vaccine composition of any one of claims 2-38, wherein at least one complementary affinity-molecule pair comprises a fusion protein comprising (i) a biotin-binding polypeptide, (ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (*P. aeruginosa* subtype B flagellin D2 domain (FlaBD2) lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 80% of the amino acid residues found in SEQ ID NO:10, and (iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:2 or SEQ ID NO:3 (*K. pneumoniae* MrkA) or an immunogenic fragment thereof comprising at least 80% of the amino acid residues found in SEQ ID NO:2 or SEQ ID NO:3.~~

~~40. The immunogenic composition or vaccine composition of any one of claims 34-39, wherein upon administration to a subject, the composition elicits antibodies that recognize native MrkA on *K. pneumoniae* expressing MrkA.~~

58~~41~~. A pharmaceutical composition comprising one or more of the immunogenic compositions or vaccine compositions of claims 1-57~~40~~ and a pharmaceutically acceptable carrier.

59~~42~~. The pharmaceutical composition of claim 58~~41~~, further comprising one or more adjuvants.

6043. The pharmaceutical composition of claim 5942, wherein the adjuvant is selected from the group of aluminum phosphate, aluminum hydroxide, phosphated aluminum hydroxide, and a TLR agonist.

6144. The pharmaceutical composition of claim 6043, wherein the TLR agonist is a TLR2, TLR4, and/or TLR5 agonist.

6245. The pharmaceutical composition of claim 6144, wherein the TLR2 agonist is a saponin or a porin.

6346. The pharmaceutical composition of claim 6245, wherein the saponin is QS21.

6447. The pharmaceutical composition of claim 6144, wherein the TLR4 agonist is monophosphoryl lipid A (MPL A).

6548. The pharmaceutical composition of claim 6144, wherein the TLR5 agonist is a flagellin.

6649. The pharmaceutical composition of any one of claims 58-6541-48, wherein the pharmaceutical composition is formulated for injection.

6750. The pharmaceutical composition of any one of claims 58-6641-49, wherein upon administration to a subject, the pharmaceutical composition elicits a Th1 and/or Th17 cell response.

6851. The pharmaceutical composition of any one of claims 58-6741-50, wherein upon administration to a subject, the pharmaceutical composition elicits an opsonic/bactericidal response against one or more of *Klebsiella*, *Pseudomonas*, and *E. coli*.

6952. The pharmaceutical composition of any one of claims 58-6841-51, wherein upon administration to a subject, the pharmaceutical composition reduces rate of transmission and/or colonization of the mucosal surfaces by one or more of *Klebsiella*, *Pseudomonas*, and *E. coli.*

70~~53~~. The pharmaceutical composition of any one of claims 58-69~~41-52~~, wherein upon administration to a subject, the pharmaceutical composition reduces rate of transmission and/or colonization of the GI tract by one or more of *Klebsiella*, *Pseudomonas*, and *E. coli*.

71~~54~~. Use of (i) an effective amount of the immunogenic composition or vaccine composition of any one of claims 1-57~~40~~, or (ii) an effective amount of the pharmaceutical composition of any one of claims 58-70~~41-53~~ in the manufacture of a medicament for immunizing a subject against *Klebsiella* infection.

72~~55~~. Use of (i) an effective amount of the immunogenic composition or vaccine composition of any one of claims 1-57~~40~~, or (ii) an effective amount of the pharmaceutical composition of any one of claims 58-70~~41-53~~ in the manufacture of a medicament for immunizing a subject against *P. aeruginosa* infection.

73~~56~~. Use of (i) an effective amount of the immunogenic composition or vaccine composition of any one of claims 1-57~~40~~, or (ii) an effective amount of the pharmaceutical composition of any one of claims 58-70~~41-53~~ in the manufacture of a medicament for immunizing a subject against *E. coli* infection.

74~~57~~. An antibody composition comprising antibodies raised in a mammal immunized with an immunogenic composition or vaccine composition of any one of claims 1-57~~40~~.

75~~58~~. A method of purifying an O-antigen polysaccharide from one or more cellular components of *Klebsiella*, wherein the cellular components include protein and/or nucleic acid, the method comprising steps of:

contacting one or more cellular components with an oxidizing reagent and an acid to obtain a mixture, wherein the pH of the mixture is between ~~about~~ 3 and 5, and wherein the oxidizing agent is sodium nitrite and the acid is acetic acid;

separating the O-antigen polysaccharide from the cellular components; and

recovering the OPS, wherein the OPS is substantially free of the other cellular components and contains a 2,5-anhydromannose residue containing a free aldehyde at its reducing terminal end.

7659. A method of making a backbone polymer comprising at least one non-biotinylated O-antigen polysaccharide, wherein the O-antigen polysaccharide comprises a primary amino group, and at least one biotinylated polysaccharide, the method comprising:

chemically linking the O-antigen polysaccharide primary amino group by mixing the O-antigen polysaccharide with a polysaccharide biotinylated and oxidized obtained by first treating the carboxylate groups of the polysaccharide with 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide N-hydroxysuccinimide (EDC) and N-Hydroxysuccinimide (NHS) and a biotin hydrazide, then treating the polysaccharide with an oxidizing agent; and reductively aminating the mixture with a reducing agent to produce BP-1.2 polymer backbone, wherein the polysaccharide is biotinylated and the chemically linked O-antigen polysaccharide is not biotinylated.

7760. A method of making a backbone polymer comprising at least one non-biotinylated O-antigen polysaccharide, wherein the O-antigen polysaccharide comprises a primary amino group, and at least one biotinylated polysaccharide, the method comprising:

chemically linking the O-antigen polysaccharide primary amino group by mixing the O-antigen polysaccharide with a polysaccharide biotinylated and CDAP-activated obtained by first treating the carboxylate groups of the polysaccharide with 1-Ethyl-3-(3-dimethylaminopropyl)-carbodiimide N-hydroxysuccinimide (EDC) and N-Hydroxysuccinimide (NHS) and a biotin hydrazide, then treating the polysaccharide with CDAP to produce BP-1.3 polymer backbone, wherein the polysaccharide is biotinylated and the chemically linked O-antigen polysaccharide is not biotinylated.

7861. A method of making a backbone polymer comprising at least one non-biotinylated O-antigen polysaccharide, wherein the O-antigen polysaccharide comprises an azido group at its reducing terminal end, and at least one biotinylated polysaccharide containing alkynes groups, the method comprising:

chemically click-linking in the presence of a catalyst the O-antigen polysaccharide containing azido groups at their reducing ends with a biotinylated polysaccharide containing alkynes,

thereby forming an OPS backbone polymer, wherein the polysaccharide is biotinylated but the chemically click-linked O-antigen polysaccharide is not biotinylated.

7962. A fusion protein comprising a biotin-binding domain and a polypeptide, wherein the fusion protein comprises an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of any one of SEQ ID NOs:16 to 26.

8063. A method of making an immunogenic complex, comprising complexing at least one backbone polymer made by the method of any one of claims 76-7859-61 with at least one of the fusion proteins of claim 7962.

8164. A fusion protein comprising:

(i) a biotin- binding protein,

(ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (P. aeruginosa FlaB flagellin D2 domain (FlaBD2) lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 80%95% of the amino acid residues found in SEQ ID NO:10, and

(iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:3 (K. pneumoniae MrkA) or an immunogenic fragment thereof comprising at least 80%95% of the amino acid residues found in SEQ ID NO:3.

8265. A fusion protein comprising:

(i) a biotin- binding protein,

(ii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:10 (P. aeruginosa flagellin subtype B D2 domain lacking the TLR5 binding motif) or an immunogenic fragment thereof comprising at least 80%95% of the amino acid residues found in SEQ ID NO:10, and

(iii) a polypeptide comprising an amino acid sequence having at least 95%, 96%, 97%, 98%, 99%, or 100% identity to the sequence of SEQ ID NO:8 (P. aeruginosa PcrV) or an immunogenic fragment thereof comprising at least ~~80%~~95% of the amino acid residues found in SEQ ID NO:8.