CLAIMS

1. A fusion protein of formula I:

$$(X)_m – (R_1)_n – A – (Y)_o – B – (Z)_p \quad \text{(formula I)}$$

wherein:

X is a signal peptide or MHHHHHH (SEQ ID NO. 2);

m is 0 or 1;

R_1 is an amino acid;

n is 0, 1, 2, 3, 4, 5 or 6;

A is Protein E from *Haemophilus influenzae* or an immunogenic fragment thereof, or PilA from *Haemophilus influenzae* or an immunogenic fragment thereof;

Y is selected from the group consisting of GG, SG, SS, GGG and $(G)_h$ wherein h is 4, 5, 6, 7, 8, 9, or 10;

o is 0 or 1;

B is PilA from *Haemophilus influenzae* or an immunogenic fragment thereof, or Protein E from *Haemophilus influenzae* or an immunogenic fragment thereof;

Z is GGHHHHHH (SEQ ID NO. 3); and

p is 0 or 1.

2. A fusion protein according to claim 1 wherein X is selected from the group consisting of FlgI, NadA and pelB.

3. A fusion protein according to any of claims 1-2 wherein m is 0.

4. A fusion protein according to any of claims 1-3 wherein n is 0.

5. A fusion protein according to any of claims 1-4 wherein A is an immunogenic fragment of Protein E, wherein Protein E is selected from any one of SEQ ID NO. 4 – SEQ ID NO. 57.

6. A fusion protein according to any of claims 1-5 wherein A is the immunogenic fragment of Protein E from *H. influenzae* as set forth in SEQ ID NO: 124.

7. A fusion protein according to any of claims 1-6 wherein Y is GG.

8. A fusion protein according to any one of claims 1-7 wherein B is an immunogenic fragment of PilA, wherein PilA is selected from any one of SEQ ID NO. 58 – SEQ ID NO. 121.

9. A fusion protein according to any of claims 1-8 wherein B is the immunogenic fragment of PilA from *H. influenzae* as set forth in SEQ ID NO. 127.

10. A fusion protein selected from the group consisting of SEQ ID NO. 136, SEQ ID NO. 138, SEQ ID NO. 140, SEQ ID NO. 142, SEQ ID NO. 144, SEQ ID NO. 146, SEQ ID NO. 148, SEQ ID NO. 150, SEQ ID NO.182, SEQ ID NO.184, SEQ ID NO.186, SEQ ID NO.188, SEQ ID NO. 190, SEQ ID NO.192, SEQ ID NO.194, SEQ ID NO.196, SEQ ID NO.198, SEQ ID NO.200, SEQ ID NO.202 and SEQ ID NO.204.

11. A fusion protein approximately 95% identical to any of SEQ ID NO. 136, SEQ ID NO. 138, SEQ ID NO. 140, SEQ ID NO. 142, SEQ ID NO. 144, SEQ ID NO. 146, SEQ ID NO. 148, SEQ ID NO. 150, SEQ ID NO. 182, SEQ ID NO. 184, SEQ ID NO. 186, SEQ ID NO. 186, SEQ ID NO. 188, SEQ ID NO. 190, SEQ ID NO. 192, SEQ ID NO. 194, SEQ ID NO. 196, SEQ ID NO. 198, SEQ ID NO. 200, SEQ ID NO. 202 or SEQ ID NO. 204.

12. A fusion protein of claim 10 or claim 11 wherein the signal peptide has been removed.

13. The fusion protein of SEQ ID NO. 148 wherein the signal peptide has been removed, SEQ ID NO. 177 (QIQKAEQN DVKLAPPTDV RSGYIRLVKN VNYYIDSESI WVDNQEPQIV HFDAVVNLDK GLYVYPEPKR YARSVRQYKI LNCANYHLTQ VRTDFYDEFW GQGLRAAPKK QKKHTLSLTP DTTLYNAAQI ICANYGEAFS VDKKGGTKKA AVSELLQASA PYKADVELCV YSTNETTNCT GGKNGIAADI TTAKGYVKSV TTSNGAITVK GDGTLANMEY ILQATGNAAT GVTWTTTCKG TDASLFPANF CGSVTQ).

14. The fusion protein of SEQ ID NO. 194 wherein the signal peptide has been removed, SEQ ID NO. 219 (IQKAEQND VKLAPPTDVR SGYIRLVKNV NYYIDSESIW VDNQEPQIVH FDAVVNLDKG LYVYPEPKRY ARSVRQYKIL NCANYHLTQV RTDFYDEFWG QGLRAAPKKQ KKHTLSLTPD TTLYNAAQII CANYGEAFSV DKKGGTKKAA

VSELLQASAP YKADVELCVY STNETTNCTG GKNGIAADIT TAKGYVKSVT TSNGAITVKG DGTLANMEYI LQATGNAATG VTWTTTCKGT DASLFPANFC GSVTQ).

15. An immunogenic composition comprising Protein E from *H. influenzae* and PilA from *H. influenzae*.

16. An immunogenic composition of claim 15 wherein Protein E is a polypeptide of SEQ ID NO. 4, a polypeptide comprising a sequence having at least 75%, 77%, 80%, 85%, 90%, 95%, 97%, 99% or 100% identity, over the entire length, to SEQ ID NO. 4, or is a polypeptide comprising an immunogenic fragment of at least 7, 10, 15, 20, 25, 30 or 50 contiguous amino acids of SEQ ID NO. 4.

17. The immunogenic composition of claim 16, wherein the immunogenic fragment comprises a B and/or T cell epitope of SEQ ID NO: 4.

18. The immunogenic composition of claims 15-17 wherein Protein E is capable of eliciting an immune response which recognizes SEQ ID NO. 4.

19. An immunogenic composition of claims 15-18 wherein PilA is a polypeptide of SEQ ID NO. 58, a polypeptide comprising a sequence having at least 80%, 85%, 90%, 95%, 97% or 100% identity, over the entire length, to SEQ ID NO. 58, or is a polypeptide comprising an immunogenic fragment of at least 7, 10, 15, 20, 25, 30 or 50 contiguous amino acids of SEQ ID NO. 58.

20. The immunogenic composition of claim 19, wherein the immunogenic fragment comprises a B and/or T cell epitope of SEQ ID NO: 58.

21. An immunogenic composition of any of claims 15-20 wherein PilA is capable of eliciting an immune response which recognizes SEQ ID NO. 58.

22. The immunogenic composition of claims 15-21, wherein the Protein E from *H. influenzae* and the PilA from *H. influenzae* are comprised as the fusion protein of claims 1-14.

23. An immunogenic composition comprising the fusion protein of SEQ ID NO. 177 (QIQKAEQN DVKLAPPTDV RSGYIRLVKN VNYYIDSESI WVDNQEPQIV HFDAVVNLDK GLYVYPEPKR YARSVRQYKI LNCANYHLTQ VRTDFYDEFW GQGLRAAPKK QKKHTLSLTP DTTLYNAAQI ICANYGEAFS VDKKGGTKKA AVSELLQASA PYKADVELCV YSTNETTNCT GGKNGIAADI TTAKGYVKSV TTSNGAITVK GDGTLANMEY ILQATGNAAT GVTWTTTCKG TDASLFPANF CGSVTQ).

24. An immunogenic composition comprising the fusion protein of SEQ ID NO. 219 (IQKAEQND VKLAPPTDVR SGYIRLVKNV NYYIDSESIW VDNQEPQIVH FDAVVNLDKG LYVYPEPKRY ARSVRQYKIL NCANYHLTQV RTDFYDEFWG QGLRAAPKKQ KKHTLSLTPD TTLYNAAQII CANYGEAFSV DKKGGTKKAA VSELLQASAP YKADVELCVY STNETTNCTG GKNGIAADIT TAKGYVKSVT TSNGAITVKG DGTLANMEYI LQATGNAATG VTWTTTCKGT DASLFPANFC GSVTQ).

25. A vaccine comprising the fusion protein of any of claims 1-14 or immunogenic compositions of any of claims 15-24.

26. Use of a therapeutically effective amount of an immunogenic composition according to any of claims 15-24 or the vaccine of claim 25 in the manufacture of a medicament for the treatment or prevention of otitis media in a subject in need thereof.

27. Use of a therapeutically effective amount of an immunogenic composition according to claim 23 or claim 24 in the manufacture of a medicament for the treatment or prevention of otitis media in a subject in need thereof.

28. Use of a therapeutically effective amount of an immunogenic composition according to any of claims 15-24 or the vaccine of claim 25 in the manufacture of a medicament for the treatment or prevention of acute exacerbation of chronic obstructive pulmonary disease (AECOPD) in a subject in need thereof.

29. Use of a therapeutically effective amount of an immunogenic composition according to claim 23 or claim 24 in the manufacture of a medicament for the treatment or prevention of acute exacerbation of chronic obstructive pulmonary disease (AECOPD) in a subject in need thereof.

30. Use of a therapeutically effective amount of an immunogenic composition according to any of claims 15-24 or the vaccine of claim 25 in the manufacture of a medicament for the treatment or prevention of pneumonia in a subject in need thereof.

31. Use of a therapeutically effective amount of an immunogenic composition according to claim 23 or claim 24 in the manufacture of a medicament for the treatment or prevention of pneumonia in a subject in need thereof.

32. Use of a therapeutically effective amount of an immunogenic composition according to any of claims 15-24 or the vaccine of claim 25 in the manufacture of a medicament for the treatment or prevention of a *H. influenzae* infection or disease in a subject in need thereof.

33. Use of a therapeutically effective amount of an immunogenic composition according to claim 23 or claim 24 in the manufacture of a medicament to be administered to a subject in need thereof for the treatment of a *H. influenzae* infection or disease.

34. The use of claim 32 or claim 33, wherein the *H. influenzae* infection or disease is an NTHi infection or disease.

35. The fusion protein of claims 1-14, or the immunogenic composition of claims 15-24, or the vaccine of claim 25, for use in the treatment or prevention of otitis media.

36. The fusion protein, the immunogenic composition, or the vaccine of claims 1-25, 35, for use in the treatment or prevention of acute exacerbations of chronic obstructive pulmonary disease (AECOPD).

37. The fusion protein, the immunogenic composition, or the vaccine of claims 1-25, 35-36, for use in the treatment or prevention of pneumonia.

38. The fusion protein, the immunogenic composition, or the vaccine of claims 1-25, 35-37, for use in the treatment or prevention of *H. influenzae* infection or disease.

39. The fusion protein, the immunogenic composition, or the vaccine of claim 38, for use in the treatment or prevention of NTHi infection or disease.

40. A process for producing periplasmic expression of a fusion protein wherein the process comprises inducing expression of proteins containing a signal peptide.

41. The process of claim 40 wherein the signal peptide is from FlgI.

42. The process of claim 40 wherein the signal peptide is from pelB.

43. The process of any one of claims 40-42 wherein the fusion protein is a fusion protein of formula (I) or the fusion protein of claims 1-14.

44. A process for making a vaccine comprising the process of claims 40-43.

Claims:

1. A fusion protein of formula 1:

$$(X)_m - (R_1)_n - A - (Y)_o - B - (Z)_p \text{ (formula I)}$$

wherein:

X is a signal peptide or MHHHHHH (SEQ ID NO. 2);

m is 0 or 1;

R_1 is an amino acid;

n is 0, 1, 2, 3, 4, 5 or 6;

A is Protein E from *Haemophilus influenzae* or an immunogenic fragment thereof, or PilA from *Haemophilus influenzae* or an immunogenic fragment thereof;

Y is selected from the group consisting of GG, SG, SS, GGG and (G)h wherein h is 4, 5, 6, 7,

8, 9, or 10;

o is 0 or 1;

B is PilA from *Haemophilus influenzae* or an immunogenic fragment thereof, or Protein E from *Haemophilus influenzae* or an immunogenic fragment thereof;

Z is GGHHHHHH (SEQ ID NO. 3); and

p is 0 or 1,

wherein when A is Protein E from *Haemophilus influenzae* or an immunogenic fragment thereof, B is not Protein E from *Haemophilus influenzae* or an immunogenic fragment thereof, and wherein when A is PilA from *Haemophilus influenzae* or an immunogenic fragment

thereof, B is not PilA from *Haemophilus influenzae* or an immunogenic fragment thereof.

2. A fusion protein according to claim 1 wherein X is selected from the group consisting of FlgI, NadA and pelB.

3. A fusion protein according to claim 1 wherein m is 0.

4. A fusion protein according to any one of claims 1-3 wherein n is 0.

5. A fusion protein according to any one of claims 1-4 wherein A is an immunogenic fragment of Protein E, wherein Protein E is selected from any one of SEQ ID NO. 4 -SEQ ID NO. 57.

6. A fusion protein according to any one of claims 1-5 wherein A is the immunogenic fragment of Protein E from *H. influenzae* as set forth in SEQ ID NO: 124.

7. A fusion protein according to any one of claims 1-6 wherein Y is GG.

8. A fusion protein according to any one of claims 1-7 wherein B is an immunogenic fragment of PilA, wherein PilA is selected from any one of SEQ ID NO. 58 – SEQ ID NO. 121.

9. A fusion protein according to any one of claims 1-8 wherein B is the immunogenic fragment of PilA from *H. influenzae* as set forth in SEQ ID NO. 127.

10. A fusion protein selected from the group consisting of SEQ ID NO. 136, SEQ ID NO. 138, SEQ ID NO. 140, SEQ ID NO. 142, SEQ ID NO. 144, SEQ ID NO. 146, SEQ ID NO. 148, SEQ ID NO. 150, SEQ ID NO.182, SEQ ID NO.184, SEQ ID NO.186, SEQ ID NO.188, SEQ ID NO. 190, SEQ ID NO.192, SEQ ID NO.194, SEQ ID NO.196, SEQ ID NO.198, SEQ ID NO.200, SEQ ID NO.202 and SEQ ID NO.204.

11. A fusion protein approximately 95% identical to any of SEQ ID NO. 136, SEQ ID NO. 138, SEQ ID NO. 140, SEQ ID NO. 142, SEQ ID NO. 144, SEQ ID NO. 146, SEQ ID NO. 148, SEQ ID NO. 150, SEQ ID NO. 182, SEQ ID NO. 184, SEQ ID NO. 186, SEQ ID NO. 186, SEQ ID NO. 188, SEQ ID NO. 190, SEQ ID NO. 192, SEQ ID NO. 194, SEQ ID NO. 196, SEQ ID NO. 198, SEQ ID NO. 200, SEQ ID NO. 202 or SEQ ID NO. 204.

12. A fusion protein of claim 10 or claim 11 wherein the signal peptide has been removed.

13. The fusion protein of SEQ ID NO. 148, wherein the signal peptide has been removed, SEQ ID NO. 177 (QIQKAEQN DVKLAPPTDV RSGYIRLVKN VNYYIDSESI WVDNQEPQIV HFDAVVNLDK GLYVYPEPKR YARSVRQYKI LNCANYHLTQ VRTDFYDEFW GQGLRAAPKK QKKHTLSLTP DTTLYNAAQI ICANYGEAFS VDKKGGTKKA AVSELLQASA PYKADVELCV YSTNETTNCT GGKNGIAADI TTAKGYVKSV TTSNGAITVK GDGTLANMEY ILQATGNAAT GVTWTTTCKG TDASLFPANF CGSVTQ).

14. The fusion protein of SEQ ID NO. 194, wherein the signal peptide has been removed, SEQ ID NO. 219 (IQKAEQND VKLAPPTDVR SGYIRLVKNV NYYIDSESIW VDNQEPQIVH FDAVVNLDKG LYVYPEPKRY ARSVRQYKIL NCANYHLTQV RTDFYDEFWG QGLRAAPKKQ KKHTLSLTPD TTLYNAAQII CANYGEAFSV DKKGGTKKAA VSELLQASAP YKADVELCVY STNETTNCTG GKNGIAADIT

TAKGYVKSVT TSNGAITVKG DGTLANMEYI LQATGNAATG VTWTTTCKGT DASLFPANFC GSVTQ).

15. An immunogenic composition comprising isolated Protein E from *H. influenzae* and isolated PilA from *H. influenzae*.

16. An immunogenic composition of claim 15 wherein Protein E is a polypeptide of SEQ ID NO. 4, a polypeptide comprising a sequence having at least 75%, 77%, 80%, 85%, 90%, 95%, 97%, 99% or 100% identity, over the entire length, to SEQ ID NO. 4, or is a polypeptide comprising an immunogenic fragment of at least 7, 10, 15, 20, 25, 30 or 50 contiguous amino acids of SEQ ID NO. 4.

17. The immunogenic composition of claim 16, wherein the immunogenic fragment comprises a B and/or T cell epitope of SEQ ID NO: 4.

18. The immunogenic composition of any one of claims 15-17 wherein Protein E is capable of eliciting an immune response which recognizes SEQ ID NO. 4.

19. An immunogenic composition of any one of claims 15-18 wherein PilA is a polypeptide of SEQ ID NO. 58, a polypeptide comprising a sequence having at least 80%, 85%, 90%, 95%, 97% or 100% identity, over the entire length, to SEQ ID NO. 58, or is a polypeptide comprising an immunogenic fragment of at least 7, 10, 15, 20, 25, 30 or 50 contiguous amino acids of SEQ ID NO. 58.

20. The immunogenic composition of claim 19, wherein the immunogenic fragment comprises a B and/or T cell epitope of SEQ ID NO: 58.

21. An immunogenic composition of any one of claims 15-20 wherein PilA is capable of eliciting an immune response which recognizes SEQ ID NO. 58.

22. The immunogenic composition of any one of claims 15-21, wherein the Protein E from *H. influenzae* and the PilA from *H. influenzae* are comprised as the fusion protein of any one of claims 1-14.

23. An immunogenic composition comprising the fusion protein of SEQ ID NO. 177 (QIQKAEQN DVKLAPPTDV RSGYIRLVKN VNYYIDSESI WVDNQEPQIV

HFDAVVNLDK GLYVYPEPKR YARSVRQYKI LNCANYHLTQ VRTDFYDEFW GQGLRAAPKK QKKHTLSLTP DTTLYNAAQI ICANYGEAFS VDKKGGTKKA AVSELLQASA PYKADVELCV YSTNETTNCT GGKNGIAADI TTAKGYVKSV TTSNGAITVK GDGTLANMEY ILQATGNAAT GVTWTTTCKG TDASLFPANF CGSVTQ).

24. An immunogenic composition comprising the fusion protein of SEQ ID NO. 219 (IQKAEQND VKLAPPTDVR SGYIRLVKNV NYYIDSESIW VDNQEPQIVH FDAVVNLDKG LYVYPEPKRY ARSVRQYKIL NCANYHLTQV RTDFYDEFWG QGLRAAPKKQ KKHTLSLTPD TTLYNAAQII CANYGEAFSV DKKGGTKKAA VSELLQASAP YKADVELCVY STNETTNCTG GKNGIAADIT TAKGYVKSVT TSNGAITVKG DGTLANMEYI LQATGNAATG VTWTTTCKGT DASLFPANFC GSVTQ).

25. A vaccine comprising the fusion protein of any one of claims 1-14 or immunogenic compositions of any one of claims 15-24.

26. The fusion protein of any one of claims 1-14, or the immunogenic composition of any one of claims 15-24, or the vaccine of claim 25, for use in the treatment or prevention of otitis media.

27. The fusion protein, the immunogenic composition, or the vaccine of any one of claims 1-25, 26, for use in the treatment or prevention of acute exacerbations of chronic obstructive pulmonary disease (AECOPD).

28. The fusion protein, the immunogenic composition, or the vaccine of any one of claims 1-25, 26-27, for use in the treatment or prevention of pneumonia.

29. The fusion protein, the immunogenic composition, or the vaccine of any one of claims 1-25, 26-28, for use in the treatment or prevention of *H. influenzae* infection or disease.

30. The fusion protein, the immunogenic composition, or the vaccine of claim 29, for use in the treatment or prevention of NTHi infection or disease.

31. A process for producing periplasmic expression of a fusion protein of claim 1 wherein the process comprises inducing expression of proteins containing a signal peptide.

32. The process of claim 31 wherein the signal peptide is from FlgI.

33. The process of claim 31 wherein the signal peptide is from pelB.

34. The process of any one of claims 31-33 wherein the fusion protein is a fusion protein of formula (1) or the fusion protein of claims 1-14.

35. A fusion protein according to claim 1 or 10-14, or an immunogenic composition according to claim 15, 23 or 24, substantially as hereinbefore described, with reference to any of the Examples.