Streptococcus pneumoniae (also called pneumococcus) is an important human pathogen accounting for significant morbidity and mortality in human and animal populations. It causes serious conditions including pneumonia, meningitis, sinusitis, and otitis media. An estimated 1.6 million people die globally from invasive pneumococcal disease each year and approximately one million of those are children. There are many different serotypes of *S. pneumoniae* (>90) distinguishable on the basis of capsule chemical structure and immunogenicity. The capsular polysaccharide is considered to be an essential virulence factor of *S. pneumoniae* as non-encapsulated strains are virtually absent among *S. pneumoniae* that are responsible for non-invasive pneumococcal disease. Capsular polysaccharides are thus used as vaccine antigens in current pneumococcal vaccines.

Current pneumococcal conjugate vaccines cover only a selected set of serotypes, (e.g. PCV7 (7 serotypes), PCV10 (10 serotypes) and PCV13 (13 serotypes)). In many populations the introduction of the PCV7 vaccine targeting serotypes 4, 6B, 9V, 14, 18C, 19F, and 23F significantly reduced the burden of pneumococcal disease. However, despite their efficacy against disease caused by targeted vaccine serotypes, serotype replacement often reduces the net effect of vaccination. For example, in a number of locations including the USA, England, Germany, Wales, and The Netherlands, serotype 19A was reported to be a common emerging non-vaccine serotype after PCV7 introduction. The emergence of non-vaccine serotypes upon the implementation of pneumococcal conjugate vaccines thus raises a problem.

In consideration of the emergence of non-vaccine serotypes associated with pneumococcal conjugate vaccines, a need exists for new streptococcal vaccines capable of inducing immunity against a broader range of serotypes.

Colonisation of the upper respiratory tract is the obligatory first step in the pathogenesis of pneumococcal disease, and therefore considered the most important risk factor for invasive pneumococcal disease. It also provides the basis for horizontal spread of pneumococci in the community, making it an important target for preventive measures.

Accordingly, a need exists for vaccines capable of inducing immunity against a broad range of pneumococcal serotypes and which are suited to upper respiratory tract administration.

Summary

The disclosure relates to an improved streptococcal vaccine that reduces or alleviates at least one deficiency of existing streptococcal vaccines.

In an aspect, there is provided the use of photon-irradiated killed streptococcal bacteria subjected to photon-radiation at energies of at least 0.01MeV in the manufacture of a medicament for preventing or treating an infection by streptococcal bacteria in a subject.

In another aspect, there is provided a vaccine composition comprising photon-irradiated killed streptococcal bacteria subjected to photon-radiation at energies of at least 0.01MeV and a pharmaceutically-acceptable excipient, diluent and/or carrier, wherein:

(i) the photon-irradiated streptococcal bacteria comprise mutant streptococcal bacteria comprising one or more defective DNA repair proteins, and/or mutant streptococcal bacteria in which at least one type of DNA repair protein is absent; and/or

(ii) the photon-irradiated streptococcal bacteria comprise modified streptococcal bacteria comprising a sequence of DNA encoding an RNA transcript, wherein the RNA transcript comprises a region of self-complementarity capable of forming a double-stranded portion after transcription.

Accordingly, the disclosure relates to at least the following embodiments:

CLAIMS

1. Use of photon-irradiated killed streptococcal bacteria subjected to photon-radiation at energies of at least 0.01MeV in the manufacture of a medicament for preventing or treating an infection by streptococcal bacteria in a subject.

2. The use according to claim 1, wherein:

(a) the medicament prevents or treats infection by a plurality of different streptococcal species and/or serotypes; and/or

(b) the photon-irradiated streptococcal bacteria comprise different: *Streptococcus* species, streptococcal serotypes, and/or streptococcal derivatives.

3. The use according to claim 1 or claim 2, wherein:

(a) the streptococcal infection comprises infection by one or more serotypes of *Streptococcus pneumoniae*; and/or

(b) the infection by streptococcal bacteria is any one or more of a respiratory tract infection, pneumonia, an ear infection, an ear-ache, a middle ear infection, otitis media, sinusitis, meningitis, conjunctivitis, bacteraemia, septicaemia, a joint infection, a bone infection, septic arthritis, sepsis, osteomyelitis, a soft tissue infection, cellulitis, myositis, periorbital cellulitis, an abscess, necrotising fasciitis, impetigo, peritonitis, a cardiac infection, endocarditis, and/or pericarditis.

4. The use according to any one of claims 1 to 3, wherein the photon-irradiated streptococcal bacteria comprise:

(a) at least one *Streptococcus pneumoniae* serotype, or at least one unencapsulated derivative of a *Streptococcus pneumoniae* serotype; and/or

(b) mutant streptococcal bacteria comprising:

(i) one or more defective DNA repair proteins; and/or

(ii) a genetic alteration causing defective DNA repair capacity.

5. The use according to claim 4, wherein the photon-irradiated mutant streptococcal bacteria comprise a defect in at least one gene that encodes a DNA repair protein.

6. The use according to claim 5, wherein the photon-irradiated mutant streptococcal bacteria are *Streptococcus pneumoniae* mutants comprising a defect in one or more genes selected from a gene encoding a DNA alkylation repair protein, a gene encoding DNA polymerase 4, *hexA*, *hexB*, *mutS*, *radC*, *recA*, *recF*, *recN*, *recO*, *uvrA*, *uvrB*, *uvrC* or *uvrD*.

7. The use according to claim 5 or claim 6, wherein the photon-irradiated mutant streptococcal bacteria comprise streptococcal Rx1 strain derivatives in which:

(i) any one or more of autolysin, hemolysin, pneumolysin, and/or PsaA protein is/are defective; and/or

(ii) any one or more of autolysin, hemolysin, and/or a PsaA protein is/are absent.

8. The use according to claim 7, wherein the photon-irradiated streptococcal Rx1 strain derivatives:

(i) comprise any one or more of a gene encoding a defective autolysin protein , a gene encoding defective hemolysin, a gene encoding defective pneumolysin, and/or a gene encoding defective PsaA protein; or

(ii) are derivatives in which any one or more of a gene encoding autolysin , a gene encoding hemolysin, and/or a gene encoding PsaA protein, is/are absent; and/or

(iii) comprise a gene encoding defective LytA protein, and a gene encoding defective pneumolysin; and/or

(iv) do not contain the *lytA* gene, and comprise a gene encoding a defective pneumolysin.

9. The use according to claim 7 or claim 8, wherein the autolysin is LytA.

10. The use according to any one of claims 1 to 9, wherein some or all of the photon-irradiated streptococcal bacteria comprise modified streptococcal bacteria comprising a sequence of DNA encoding an RNA transcript, wherein the RNA transcript comprises a

region of self-complementarity capable of forming a double-stranded portion after transcription.

11. The use according to claim 10, wherein the portion of the double-stranded RNA transcript is at least: 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or 70 base pairs in length.

12. The use according to any one of claims 1 to 11, wherein the photon-irradiated streptococcal bacteria comprise:

(a) auxotrophic mutant streptococcal bacteria; and/or

(b) at least one recombinant DNA portion encoding an antigen or a component thereof that:

(i) inactivates or attenuates the bacteria; and/or

(ii) induces or enhances an immune response in the subject.

13. The use according to claim 12, wherein the recombinant DNA portion replaces or disrupts an endogenous gene necessary for pathogenicity, infection, multiplication, growth *in vivo*, or any combination thereof.

14. The use according to any one of claims 1 to 13, wherein:

(a) the photon-irradiated streptococcal bacteria comprise whole killed streptococcal bacteria; and/or

(b) the photon-irradiated streptococcal bacteria are to be administered to the subject mucosally or intranasally; and/or

(c) the photon-irradiated streptococcal bacteria are to be administered to the subject in combination with an adjuvant; and/or

(d) one, two or three separate doses of the photon-irradiated streptococcal bacteria are to be administered to the subject; and/or

(e) the subject is a human; and/or

(f) the photon-irradiated streptococcal bacteria are to be administered a total dosage of at least: 8 kilogray (kGy), 9kGy, 10kGy, 11kGy, 12kGy, 15kGy, 20kGy, 25kGy, 30 kGy, 40kGy, or 50 kGy of photon-irradiation.

15. The use according to any one of claims 1 to 14, wherein the photon-irradiated streptococcal bacteria comprise gamma-irradiated streptococcal bacteria, X-irradiated streptococcal bacteria, or a combination thereof.

16. The method according to any one of claims 1 to 15, wherein the photon-irradiated streptococcal bacteria comprise streptococcal Rx1 strain derivatives:

(i) comprise a gene encoding a defective LytA protein, a gene encoding defective pneumolysin, and a gene encoding defective PsaA protein;

(ii) comprise a gene encoding a defective LytA protein, a gene encoding defective pneumolysin, and in which a gene encoding PsaA protein is absent;

(iii) comprise a gene encoding a defective PsaA protein, a gene encoding defective pneumolysin, and in which a gene encoding LytA protein is absent; or

(iv) comprise a gene encoding defective pneumolysin, in which a gene encoding LytA protein is absent, and in which a gene encoding PsaA protein is absent.

17. A vaccine composition comprising photon-irradiated killed streptococcal bacteria subjected to photon-radiation at energies of at least 0.01MeV and a pharmaceutically-acceptable excipient, diluent and/or carrier, wherein:

(i) the photon-irradiated streptococcal bacteria comprise mutant streptococcal bacteria comprising one or more defective DNA repair proteins, and/or mutant streptococcal bacteria in which at least one type of DNA repair protein is absent; and/or

(ii) the photon-irradiated streptococcal bacteria comprise modified streptococcal bacteria comprising a sequence of DNA encoding an RNA transcript, wherein the RNA transcript comprises a region of self-complementarity capable of forming a double-stranded portion after transcription.

18. The vaccine composition according to claim 17, wherein:

(a) the mutant streptococcal bacteria are *Streptococcus pneumoniae* mutants comprising a defect in one or more genes selected from. a gene encoding a DNA alkylation repair protein, a gene encoding DNA polymerase 4, *hexA*, *hexB*, *mutS*, *radC*, *recA*, *recF*, *recN*, *recO*, *uvrA*, *uvrB*, *uvrC* or uvrD; and/or

(b) the modified streptococcal bacteria are modified *Streptococcus pneumoniae* bacteria and the portion of the double-stranded RNA transcript is at least. 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65 or 70 base pairs in length; and/or

(c) the photon-irradiated streptococcal bacteria comprise at least one recombinant DNA portion encoding an antigen or a component thereof that:

(i) inactivates or attenuates the bacteria; or

(ii) induces or enhances an immune response in the subject; and/or

(d) the photon-irradiated streptococcal bacteria comprise streptococcal Rx1 strain derivative strains in which:

(i) any one or more of autolysin, hemolysin, pneumolysin, and/or PsaA protein is/are defective; and/or

(ii) any one or more of autolysin, hemolysin, and/or a PsaA protein is/are absent.

(e) the photon-irradiated mutant streptococcal bacteria comprise streptococcal Rx1 strain derivatives which:

(i) comprise a gene encoding a defective LytA protein, a gene encoding defective pneumolysin, and a gene encoding defective PsaA protein;

(ii) comprise a gene encoding a defective LytA protein, a gene encoding defective pneumolysin, and in which a gene encoding PsaA protein is absent;

(iii) comprise a gene encoding a defective PsaA protein, a gene encoding defective pneumolysin, and in which a gene encoding LytA protein is absent; or

(iv) comprise a gene encoding defective pneumolysin, in which a gene encoding LytA protein is absent, and in which a gene encoding PsaA protein is absent.

19. The vaccine composition according to claim 17 or claim 18, wherein the photon-irradiated streptococcal bacteria comprise X-irradiated mutant streptococcal bacteria and/or X-irradiated modified streptococcal bacteria.

20. A method for preparing the vaccine composition according to any one of claims 17 to 19, the method comprising:

(i) photon-irradiating a preparation of streptococcal bacteria with photon-radiation at energies of at least 0.01MeV to thereby kill the bacteria; and

(ii) combining the photon-irradiated streptococcal bacteria with a pharmaceutically-acceptable excipient, diluent and/or carrier.