TITLE: EXPRESSION AND PURIFICATION OF THE α-HELIX REGION OF PspA (PNEUMOCOCCAL SURFACE PROTEIN A) VARIANTS

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ABSTRACT:

Streptococcus pneumoniae is responsible for diseases such as otitis media, pneumonia and meningitis. The polysaccharide conjugate vaccines are based on the induction of antibodies against the capsular polysaccharide and have proved to be very effective in preventing invasive infections caused by pneumococci in children. Unvaccinated individuals also showed a decrease in pneumococcal disease due to herd effect. However, there was a rapid replacement of nasopharyngeal colonization and disease by non-vaccine serotypes. The elderly remain a particularly affected group, with a high incidence of pneumococcal pneumonia. Pneumococcal surface protein A (PspA) is a virulence factor found in all pneumococcal isolates. This protein acts on the interaction between the pathogen and the host by interfering in the activation and deposition of factors of the complement system on the surface of the bacterium. Its structure has a choline-binding domain at the C-terminal region; next to this domain is a proline-rich region and at the N-terminal region there is an α-helix domain that is exposed on the bacterial surface. There is variability between strains in the α-helix region and the protein is classified in 6 clades. It is not known if the decrease in the immunity to any specific pneumococcal antigen would be related to the high susceptibility of the elderly to pneumococcal pneumonia. Previous work has shown evidence that lower levels of anti-PspA antibodies might be related to this increased susceptibility. This project aims to purify the mature N-terminal α-helix region of PspA from clades 1 to 6. These recombinant proteins will be used to assess whether there are differences in levels of anti-PspA antibodies in the serum of young adults and the elderly and whether there is any correlation between antibody levels and protection against colonization challenge. The N-terminal α-helix regions of clades 1 to 6 were amplified by PCR using a high fidelity Taq polymerase, subcloned into the pGEM-TEasy vector and subsequently cloned into the pAE vector for expression in *Escherichia coli* with a polyhistidine tag. PspA1α, PspA2α and PspA3α were expressed in *E. coli* BL21 Star DE3 pLysS and purified by nickel affinity chromatography. These proteins were obtained with high yield and high purity. From an initial culture of 300 mL, 18 mg of PspA1α, 28 mg of PspA2α and 28 mg of PspA3α were obtained. PspA4α, PspA5α and PspA6α are currently being purified.

Keywords: *Streptococcus pneumoniae*, pneumonia, PspA

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