Evaluation of live attenuated *Streptococcus pneumoniae* vaccine strains on the epithelial response to colonisation using a human challenge model

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Background: *Streptococcus pneumoniae* frequently colonises the human nasopharynx but causes over 500,000 deaths each year from Pneumonia, Sepsis and Meningitis. Nasopharyngeal carriage is required for transmission and is a pre-requisite for disease. The conjugate polysaccharide vaccines have proven effective in decreasing disease. However, replacement of vaccine serotypes with non-vaccine types in carriage threatens the future of the vaccines' efficiency.

Using an Experimental Human Pneumococcal Challenge model (EHPC) and epithelial cell culture models, we have previously shown that pneumococcal colonisation involves both direct epithelial association and micro-invasion, inducing innate immunity and clearance without overt disease. Repeated challenge in the EHPC with the same strain decreases subsequent carriage efficiency and diminishes transmission potential and/or progression to disease, suggesting active mucosal immunity in the nasopharynx.

Methods and materials: We have generated live attenuated strains of 6B *S. pneumoniae* (AS1 and AS2) that have double virulence deletions and cannot revert to cause disease. Here, we have explored the hypothesis that despite their attenuation in a mouse model of disease, these attenuated strains retain their ability to invade the epithelium and induce epithelial-derived innate immunity in humans.

Colonisation were measured by confocal microscopy and microbiology density by CFU counts. Epithelial activation was measured by flow cytometry, ELISA and RNAseq.

Results: We found that both mutants colonised the human nasopharynx and formed epithelial associations with micro-invasion in the EHPC model. *In vitro,* both mutants adhered, invaded and transmigrated across the epithelium 4-fold less than wild type. However, both mutants still resulted in secretion of IL-8, IL-6 and ICAM-1 secretion and barrier integrity was maintained. PCA analyses revealed that epithelial transcriptomic responses between wild type and the mutants generally overlapped, indicating overall similar stimulation of signaling pathways following exposure.

Conclusion: The results reveal that attenuation of these pneumococcal strains has not led to loss of their ability to elicit a mucosal immune/inflammatory response. This approach provides an exciting new pipeline for the development and testing of novel vaccines. The application of these attenuated strains in the EHPC also has the potential to provide important new knowledge on the mechanisms behind bacterial clearance, transmission and disease progression during colonisation.

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Do phenotypic and genotypic characteristics of uropathogenic *E. coli* isolates differ in acute and recurrent phases of urinary tract infection?

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Background: Urinary tract infection (UTI) is one of the most common infections worldwide, and uropathogenic *Escherichia coli* (UPECs) are the main causative pathogens. However, their characteristics may vary depend on the phase (acute or recurrent) of UTI.

This study was conducted to evaluate any probable differences between UPEC isolates in acute and recurrent phases of UTI in the same patient.

Methods and materials: The urine samples of patients suffering from UTI in two referral (Loghman and Imam) hospitals of Tehran, Iran were collected and the patients were under supervision for any probable recurrence during 6 months. The isolates were subjected for differential cultures to select *E. coli* strains. Then, phylo- and sero-grouping, antibiotic susceptibility test, biofilm formation assay was performed, and the genes responsible for antibiotic resistance, biofilm formation and iron transferring were detected.

Results: Sixty isolates (one isolate from each phase) were selected from 30 patients (16 females, 14 males) at different ages. In two-third of patients, phylogroups of the *E. coli* isolates were identical in the acute and recurrent phases, 70% of them were B2/B2. Similarly, in 70% of the patients, serogroups of the isolates were alike in both phases, 85.7% of them have O25/O25 pattern. The isolates with O15 serotype were significantly seen more in recurrent phase. However, only one-third of the isolates showed equal biofilm intensity in both phases (most of them were weak/weak). The isolates with strong biofilm ability were mostly detected in recurrent phase. When comparing antibiotic resistance pattern of the isolates in two different phases, the isolates from Imam hospital had more antibiotics in common to be resistant to than the ones from Loghman hospital. Likewise, the isolates in Imam hospital had more antibiotic resistance, biofilm-related and iron transfer genes in common than the ones in Loghman hospital. Interestingly, distribution of two resistance genes, *sul1* and *qnrA*, was more predominant in Imam and Loghman hospitals respectively.

Conclusion: Although it seems that UPEC isolates that are responsible for acute or recurrent phases of UTI may belong to common phylo- or sero-groups, but they show different resistance and virulence characteristics. These characteristics may vary from hospital to hospital.

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