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PROGRAMME & ABSTRACTS

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The role of helicases in the processing and recovery of DNA replication forks following replication arrest in living *Escherichia coli* cells

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DNA replication is essential for all forms of life and must be completed accurately for the duplication of a cell. Despite being a precise and rapid process, the replisome is often impeded by barriers, arresting the progression of replication and potentially threatening genome integrity. Such arrest may lead to the dissociation of replisome components and the collapse of the replication fork. A major source of replication fork stalling is the encounter of the replisome with proteins bound to DNA. In *E. coli*, a multitude of recombination proteins have been implicated in the processing of arrested replication forks, including several helicases that may reverse the stalled fork into a Holliday junction. In this study, the early processes that a cell undergoes to restore a replication fork structure have been assessed. The main focus has been to investigate the significant helicases RecG, RuvABC and RecQ for their roles in DNA repair and fork restoration.

In order to achieve this, an *in vivo* repressor/operator system has been established to create a site-specific nucleoprotein replication block in the *E. coli* chromosome. The addition of temperature sensitive replisome components can be used to induce replication fork collapse. The formation of the replication block and its subsequent release can be visualised using fluorescence microscopy, cell viability assays and 2-dimensional agarose gel electrophoresis to analyse the DNA structures present at the nucleoprotein blockage and subsequently evaluate the effect of deleting key helicases on replication fork processing and recovery. A novel role of RecQ has been elucidated and the relative contributions of RecG and RuvABC to fork and HJ processing have been determined. These findings alter the current perception of the helicase roles in fork recovery following collapse.

Biofilm dormancy enhances antimicrobial tolerance in *S. epidermidis*

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Indwelling medical devices have been increasingly used in modern medicine and have saved millions of lives worldwide. However, they can also be an important source of infections, most commonly caused by coagulase negative-staphylococci, particularly by biofilm forming *Staphylococcus epidermidis*. A key feature of biofilms is its enhanced tolerance to antibiotics. Several mechanisms have been proposed to contribute to this phenomenon. We recently developed an in vitro model able to stimulate the induction or prevention of biofilm dormancy. Herein, we used that model to determine if biofilms with induced dormancy presented a distinct antimicrobial tolerance profile than biofilms with prevented dormancy. Both clinical or commensal isolates where included and a total of 43 unique isolates, from different parts of the world were tested. Biofilms were exposed to tetracycline, vancomycin and rifampicin and where analysed by flow citometry, CFU counts and CLSM. Three unique observations were obtained. First, biofilm dormancy was found as a widespread condition in both clinical and commensal isolates, suggesting this is a fundamental process not only related to the infectious process. Second, while vancomycin did not presented any significant effect on the tested biofilms, tetracycline and rifampicin significantly reduced the number of CFUs in biofilms with prevented dormancy tested (up to 4 log killing under 8 h), but were significantly less effective in biofilms with induced dormancy. The third and more curious observation was that the very high reduction in cultivable bacteria was not correlated with the reduction of total and viable cells. Overall, our data suggests in one hand that biofilms with induced dormancy are more tolerant to tetracycline and rifampicin and that those antibiotics further induce dormancy in biofilms, instead of effective eliminating the biofilm bacteria.

Prevalence of soil-borne zoonotic pathogens of public health significance in Punjab province, Pakistan

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**Introduction:** Utilizing molecular detection assays, surveillance for Extremely Dangerous Pathogens (EDPs) from a complex ecological niche (soil) is imperative to devise appropriate interventions for public and animal health. Such an approach is important for a particular setting such as Pakistan where appropriate bio-containment facilities for culturing and archiving EDPs are not available.

**Methods:** We conducted a cross-sectional study to estimate genomic prevalence of *Burkholderia mallei*, *Coxiella burnetii*, *Francisella tularensis*, and *Bacillus anthracis* in soil representing select districts (n=69) of Punjab province, Pakistan. The study included 485 villages of districts Lahore (n=29), Sheikhpura (n=295), Gujranwala (n=360), Faisalabad (n=370), Sahiwal (n=255), Attock (n=225), Sargodha (n=370), Chakwal (n=190) and DG Khan (n=215). A total of 2,425 soil samples representing different geographical locations were processed; 05 were collected from each village comprising of 04 from different livestock barns and 01 from a nearby agriculture land with no apparent animal and human interaction. Genomic DNA was extracted and processed using well-optimized and validated molecular assay (real time PCR) for chromosomal gene of *B. mallei*, transposase gene (ISIII) for *C. burnetii*, lipoprotein gene (Tul4) for *F. Tularensis*, and both capsular (CapB) and protective antigen (PA) for *B. anthracis*. 

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