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Carbapenems-resistant Enterobacteriaceae infections are increasing worldwide representing an emerging public health problem. The application of phylogenetic and phylodynamic analyses to bacterial whole-genome sequencing data have become essential in the epidemiological surveillance of multi-drug-resistant nosocomial pathogens. Between January 2012 and February 2013, twenty-one multi-drug-resistant *K. pneumoniae* strains, were collected from patients hospitalized among different wards of the University Hospital Campus Bio-Medico. Epidemiological contact tracing of patients and Bayesian phylogenetic analysis of bacterial whole-genome sequencing data were used to investigate the evolution and spatial dispersion of *K. pneumoniae* in support of hospital infection control. The epidemic curve of incident *K. pneumoniae* cases showed a bimodal distribution of cases with two peaks separated by forty-six days between November 2012 and January 2013. The time-scaled phylogeny suggested that *K. pneumoniae* strains isolated during the study period may have been introduced into the hospital setting as early as 2007. Moreover, the phylogeny showed two different epidemic introductions in 2008 and 2009. Bayesian genomic epidemiology is a powerful tool that promises to improve the surveillance and control of multi-drug-resistant pathogens in an effort to develop effective infection prevention in health-care settings or constant strains reintroduction.

#### **A67 Use of next-generation whole-genome sequencing to understand drug-resistant *Mycobacterium tuberculosis* in Botswana**

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The need to curb the emergence and spread of *M. tuberculosis* drug resistance requires exploration of new methods especially in high incidence countries. Effective treatment informed by timely and accurate drug susceptibility testing is critical to control drug-resistant tuberculosis. Next-generation whole-genome sequencing is increasingly becoming attractive and could potentially provide a fast and comprehensive determination of drug susceptibility that could inform timely treatment decisions. The aim of this study is to use next-generation whole-genome sequencing analysis to understand *M. tuberculosis* drug resistance, population structure, and transmission dynamics in Botswana. The study will be a retrospective cross sectional

study using isolates from adults diagnosed with drug-resistant pulmonary tuberculosis by culture based drug susceptibility testing at Botswana National Tuberculosis Reference Laboratory in Gaborone, Botswana. A total of 150 isolates with a spectrum of resistance ranging from mono-resistance to multidrug-resistant tuberculosis (MDR-TB) will be selected for next-generation whole-genome sequencing using the Illumina MiSeq<sup>®</sup> system (Illumina, San Diego, California). FASTQ files or Unmapped Binary Alignment Map (BAM) files of the reads generated will be assembled and aligned using Geneious, Genome Analysis Toolkit (GATK), and SamTools. Alignment output for each sample will then be indexed, sorted, and merged into a single alignment file using SamTools. SNP calling will be done using Genius, GATK, and SamTools; the SNPs detected by at least two methods will be deemed real and compared to a TB drug resistance mutation list (<http://pfgrc.jcvi.org/>). The list includes 1,086 SNPs identified in thirty *M. tuberculosis* genes.

#### **A68 Bats as a source of zoonotic spillover: Investigating viruses in enteric bat samples from Viet Nam**

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Bats, belonging to the order Chiroptera, are the natural reservoir hosts for an array of zoonotic viruses. Aspects of bat ecology, behavior, and physiology, such as migrating great distances, roosting in close association in large numbers and variation in metabolic rate and core body temperature during sustained flight, make them a unique concern for viral zoonotic transfer. As part of the Viet Nam Initiative on Zoonotic Infections (VIZIONS) project, 169 enteric bat samples were collected from two sites in the Dong Thap province in Viet Nam, ~100 km apart, and Illumina sequenced at the Sanger Institute. Based on host mtDNA sequence present in these enteric samples, we identified *Scotophilus kuhlii* as the host species for >97 per cent of the 196 samples, with remaining samples of the *Myotis*, *Murina*, or *Pipistrellus* genera. Significant quantities of Alphacoronavirus, Rotavirus, and Mamastrovirus reads were identified in the enteric bat samples using Kraken. We confirmed significant mixing and jumping of Alphacoronavirus between the two locations, using two independent analyses: Bayesian Tip-associated Significance testing, which confirms no significant clustering of virus with respect to location, and host-state reconstruction analysis, which predicted a mean of seventeen host-jumps between the two locations. These findings suggest that 100 km is negligible for *Scotophilus kuhlii* in terms of viral transfer.