Direct whole-genome sequencing of Plasmodium falciparum specimens from dried erythrocyte spots

Background: Plasmodium falciparum malaria remains a major health burden and genomic research represents one of the necessary approaches for continued progress towards malaria control and elimination. Sample acquisition for this purpose is troublesome, with the majority of malaria-infected individuals living in rural areas, away from main infrastructure and the electrical grid. The aim of this study was to describe a low-tech procedure to sample P. falciparum specimens for direct whole genome sequencing (WGS), without use of electricity and cold-chain. Methods: Venous blood samples were collected from malaria patients in Bandim, Guinea-Bissau and leukocyte-depleted using Plasmodipur filters, the enriched parasite sample was spotted on Whatman paper and dried. The samples were stored at ambient temperatures and subsequently used for DNA-extraction. Ratios of parasite:human content of the extracted DNA was assessed by qPCR, and five samples with varying parasitaemia, were sequenced. Sequencing data were used to analyse the sample content, as well as sample coverage and depth as compared to the 3d7 reference genome. Results: qPCR revealed that 73% of the 199 samples were applicable for WGS, as defined by a minimum ratio of parasite:human DNA of 2:1. WGS revealed an even distribution of sequence data across the 3d7 reference genome, regardless of parasitaemia. The acquired read depths varied from 16 to 99×, and coverage varied from 87.5 to 98.9% of the 3d7 reference genome. SNP-analysis of six genes, for which amplicon sequencing has been performed previously, confirmed the reliability of the WGS-data. Conclusion: This study describes a simple filter paper based protocol for sampling P. falciparum from malaria patients for subsequent direct WGS, enabling acquisition of samples in remote settings with no access to electricity.
Draft Genome Sequence of Acinetobacter johnsonii C6, an Environmental Isolate Engaging in Interspecific Metabolic Interactions

Acinetobacter johnsonii C6 originates from creosote-polluted groundwater and performs ecological and evolutionary interactions with Pseudomonas putida in biofilms. The draft genome of A. johnsonii C6 is 3.7 Mbp and was shaped by mobile genetic elements. It reveals genes facilitating the biodegradation of aromatic hydrocarbons and resistance to antimicrobials and metals.

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Authors: Kaas, R. S. (Intern), Mordhorst, H. (Intern), Leekitcharoenphon, P. (Intern), Jensen, J. D. (Intern), Haagensen, J. A. J. (Intern), Molin, S. (Intern), Pamp, S. J. (Intern)
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Genomic Dissection of Travel-Associated Extended-Spectrum-Beta-Lactamase-Producing Salmonella enterica Serovar Typhi Isolates Originating from the Philippines: a One-Off Occurrence or a Threat to Effective Treatment of Typhoid Fever?

One unreported case of extended-spectrum-beta-lactamase (ESBL)-producing Salmonella enterica serovar Typhi was identified, whole-genome sequence typed, among other analyses, and compared to other available genomes of S. Typhi. The reported strain was similar to a previously published strain harboring blaSHV-12 from the Philippines and likely part of an undetected outbreak, the first of ESBL-producing S. Typhi.

**General information**

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Genomic Signature of Multidrug-Resistant Salmonella enterica Serovar Typhi Isolates Related to a Massive Outbreak in Zambia between 2010 and 2012.

Retrospectively, we investigated the epidemiology of a massive Salmonella enterica serovar Typhi outbreak in Zambia during 2010 to 2012. Ninety-four isolates were susceptibility tested by MIC determinations. Whole-genome sequence...
typing (WGST) of 33 isolates and bioinformatic analysis identified the multilocus sequence type (MLST), haplotype, plasmid replicon, antimicrobial resistance genes, and genetic relatedness by single nucleotide polymorphism (SNP) analysis and genomic deletions. The outbreak affected 2,040 patients, with a fatality rate of 0.5%. Most (83.0%) isolates were multidrug resistant (MDR). The isolates belonged to MLST ST1 and a new variant of the haplotype, H58B. Most isolates contained a chromosomally translocated region containing seven antimicrobial resistance genes, catA1, blaTEM-1, dfrA7, sul1, sul2, strA, and strB, and fragments of the incompatibility group Q1 (IncQ1) plasmid replicon, the class 1 integron, and the mer operon. The genomic analysis revealed 415 SNP differences overall and 35 deletions among 33 of the isolates subjected to whole-genome sequencing. In comparison with other genomes of H58, the Zambian isolates separated from genomes from Central Africa and India by 34 and 52 SNPs, respectively. The phylogenetic analysis indicates that 32 of the 33 isolates sequenced belonged to a tight clonal group distinct from other H58 genomes included in the study. The small numbers of SNPs identified within this group are consistent with the short-term transmission that can be expected over a period of 2 years. The phylogenetic analysis and deletions suggest that a single MDR clone was responsible for the outbreak, during which occasional other S. Typhi lineages, including sensitive ones, continued to cocirculate. The common view is that the emerging global S. Typhi haplotype, H58B, containing the MDR IncHI1 plasmid is responsible for the majority of typhoid infections in Asia and sub-Saharan Africa; we found that a new variant of the haplotype harboring a chromosomally translocated region containing the MDR islands of IncHI1 plasmid has emerged in Zambia. This could change the perception of the term “classical MDR typhoid” currently being solely associated with the IncHI1 plasmid. It might be more common than presently thought that S. Typhi haplotype H58B harbors the IncHI1 plasmid or a chromosomally translocated MDR region or both.

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Spread of Extended Spectrum Cephalosporinase-Producing Escherichia coli Clones and Plasmids from Parent Animals to Broilers and to Broiler Meat in a Production Without Use of Cephalosporins

Objectives: This study investigated the occurrence of extended spectrum cephalosporinase (ESC)–producing Escherichia coli in a broiler production with no cephalosporin use and a low use of antimicrobials in general. Furthermore, it investigated whether the current consumption of aminopenicillins selects for ESC-producing E. coli and whether certain clones or plasmids spread from imported parent flocks to the meat. Materials and Methods: ESC-producing E. coli was isolated using MacConkey broth with 1 mg/L of ceftriaxone. ESC genes were identified using polymerase chain reaction and sequencing. Isolates with blaCMY-2 were subtyped by pulsed-field gel electrophoresis (PFGE), phylotyping, and antimicrobial susceptibility testing. Selected isolates were used as donors in filter-mating experiments, multilocus sequence typing (MLST), and plasmid replicons were typed. Aminopenicillin use at the farm (not flock) level was obtained from VetStat, a database for mandatory registration of veterinary prescriptions in Denmark. Results: ESC-producing E. coli occurred in 93% (27/29) of broiler parent farms in 2011, 27% (53/197) of broiler flocks in 2010, and 3.3% (4/121) of Danish retail broiler meat in 2009 and 8.6% (16/187) in 2010. The ESC producing E. coli contained blaCMY-2, blaSHV-2 or blaCTX-M-1. Isolates with blaCMY-2 represented 35 PFGE groups. One group dominated (39 isolates) and included isolates with indistinguishable PFGE patterns from parents, broilers, and meat. Most blaCMY-2 isolates were susceptible to non-β-lactams, and blaCMY-2 was mostly present on horizontally transferable IncI1 or IncK plasmids. Phylogroup D was most common and E. coli MLST types previously found in humans were observed. Broiler farms with registered aminopenicillin use had significantly higher occurrence of ESC E. coli. Conclusions: ESC-producing E. coli from flocks of imported broiler parents spread clonally and horizontally to broiler meat (including potentially human pathogenic types) even in a country with no cephalosporin use. Use of aminopenicillins may influence the persistence of ESC-producing E. coli in the broiler production, but other factors should be investigated.

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