Publications:

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.
CHTyper, a Web Tool for Subtyping of Extraintestinal Pathogenic Escherichia coli based on the fumC and fimH Alleles

Escherichia coli can cause a variety of extra-intestinal infections, such as urinary tract infection, meningitis, peritonitis and septicemia....

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Organisations: Department of Bio and Health Informatics, Genomic Epidemiology, Research Group for Genomic Epidemiology, Statens Serum Institute, University of Washington
SCCmecFinder, a Web-Based Tool for Typing of Staphylococcal Cassette Chromosome mec in Staphylococcus aureus Using Whole-Genome Sequence Data

Typing of methicillin-resistant Staphylococcus aureus (MRSA) is important in infection control and surveillance. The current nomenclature of MRSA includes the genetic background of the S. aureus strain determined by multilocus sequence typing (MLST) or equivalent methods like spa typing and typing of the mobile genetic element staphylococcal cassette chromosome mec (SCCmec), which carries the mecA or mecC gene. Whereas MLST and spa typing are relatively simple, typing of SCCmec is less trivial because of its heterogeneity. Whole-genome sequencing (WGS) provides the essential data for typing of the genetic background and SCCmec, but so far, no bioinformatic tools for SCCmec typing have been available. Here, we report the development and evaluation of SCCmecFinder for characterization of the SCCmec element from S. aureus WGS data. SCCmecFinder is able to identify all SCCmec element types, designated I to XIII, with subtyping of SCCmec types IV (2B) and V (5C2). SCCmec elements are characterized by two different gene prediction approaches to achieve correct annotation, a Basic Local Alignment Search Tool (BLAST)-based approach and a k-mer-based approach. Evaluation of SCCmecFinder by using a diverse collection of clinical isolates (n = 93) showed a high typeability level of 96.7%, which increased to 98.9% upon modification of the default settings. In conclusion, SCCmecFinder can be an alternative to more laborious SCCmec typing methods and is freely available at https://cge.cbs.dtu.dk/services/SCCmecFinder. IMPORTANCE SCCmec in MRSA is acknowledged to be of importance not only because it contains the mecA or mecC gene but also for staphylococcal adaptation to different environments, e.g., in hospitals, the community, and livestock. Typing of SCCmec by PCR techniques has, because of its heterogeneity, been challenging, and whole-genome sequencing has only partially solved this since no good bioinformatic tools have been available. In this article, we describe the development of a new bioinformatic tool, SCCmecFinder, that includes most of the needs for infection control professionals and researchers regarding the interpretation of SCCmec elements. The software detects all of the SCCmec elements accepted by the International Working Group on the Classification of Staphylococcal Cassette Chromosome Elements, and users will be prompted if diverging and potential new elements are uploaded. Furthermore, SCCmecFinder will be curated and updated as new elements are found and it is easy to use and freely accessible.

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The aim of this study was to construct a valid publicly available method for in silico fimH sub-typing of Escherichia coli particularly suitable for differentiation of fine-resolution subgroups within clonal groups defined by standard multi-locus sequence typing (MLST). FimTyper was constructed as a FASTA database containing all currently known fimH alleles. The software source code is publicly available on https://bitbucket.org/genomicepidemiology/fimtyper, the database freely available at https://bitbucket.org/genomicepidemiology/fimtyper_db, and a service implementing the software available at https://cge.cbs.dtu.dk/services/FimTyperFimTyper was validated on three datasets; (i) containing Sanger sequences of fimH alleles of 42 E. coli isolates generated prior to the current study, (ii) whole-genome sequence data of 243 third-generation cephalosporins-resistant E. coli isolates, and (iii) a randomly chosen subset of 40 E. coli isolates from dataset (ii), which were subjected to conventional fimH sub-typing. The combination of the three datasets enabled an evaluation and comparison of FimTyper on both Sanger sequences and WGS data. FimTyper correctly predicted all 40 fimH subtypes from the Sanger sequences from dataset (i), and successfully analyzed all 243 drafted genomes from dataset (ii). FimTyper sub-typing of the Sanger sequences and WGS data from dataset (iii) were in complete agreement. Additionally, fimH sub-typing was evaluated on a phylogenetic network of 122 ST131 E. coli isolates. There were perfect concordance between the typology and fimH-based sub-clones within ST131 with accurate identification of the pandemic multidrug resistant clonal subgroup ST131-H30. FimTyper provides a standardized tool, as a rapid alternative to conventional fimH sub-typing, highly suitable for surveillance and outbreak detection.
PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens

Background
Antibiotic resistance is a major health problem, as drugs that were once highly effective no longer cure bacterial infections. WGS has previously been shown to be an alternative method for detecting horizontally acquired antimicrobial resistance genes. However, suitable bioinformatics methods that can provide easily interpretable, accurate and fast results for antimicrobial resistance associated with chromosomal point mutations are still lacking.

Methods
Phenotypic antimicrobial susceptibility tests were performed on 150 isolates covering three different bacterial species: Salmonella enterica, Escherichia coli and Campylobacter jejuni. The web-server ResFinder-2.1 was used to identify acquired antimicrobial resistance genes and two methods, the novel PointFinder (using BLAST) and an in-house method (mapping of raw WGS reads), were used to identify chromosomal point mutations. Results were compared with phenotypic antimicrobial susceptibility testing results. Results
A total of 685 different phenotypic tests associated with chromosomal resistance to quinolones, polymyxin, rifampicin, macrolides and tetracyclines resulted in 98.4% concordance. Eleven cases of disagreement between tested and predicted susceptibility were observed: two C. jejuni isolates with phenotypic fluoroquinolone resistance and two with phenotypic erythromycin resistance and five colistin-susceptible E. coli isolates with a detected pmrB V161G mutation when assembled with Velvet, but not when using SPAdes or when mapping the reads.

Conclusions
PointFinder proved, with high concordance between phenotypic and predicted antimicrobial susceptibility, to be a user-friendly web tool for detection of chromosomal point mutations associated with antimicrobial resistance.

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