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.
Prevalence and risk factors for CTX-M gram-negative bacteria in hospitalized patients at a tertiary care hospital in Kilimanjaro, Tanzania

Emergence and spread of extended spectrum beta-lactamase (ESBL)-producing gram-negative bacteria, mainly due to CTX-M, is a major global public health problem. Patients infected with ESBL-producing gram-negative bacteria have an increased risk of treatment failure and death. We investigated the prevalence and risk factors for CTX-M gram-negative bacteria isolated from clinical specimens of patients hospitalized at a tertiary care hospital in Kilimanjaro, Tanzania. Isolated gram-negative bacteria from inpatients admitted at Kilimanjaro Christian Medical Centre (KCMC) between August 2013 and August 2015 were fully genome sequenced. The prevalence of ESBL-producing gram-negative bacteria was determined based on the presence of bla_{CTX-M}. The odds ratio (OR) and risk factors for ESBL-producing gram-negative bacteria due to CTX-M were assessed using logistic regression models. The overall CTX-M prevalence (95% CI) was
13.6% (10.1–18.1). Adjusted for other factors, the OR of CTX-M gram-negative bacteria for patients previously hospitalized was 0.26 (0.08–0.88), p = 0.031; the OR for patients currently on antibiotics was 4.02 (1.29–12.58), p = 0.017; the OR for patients currently on ceftriaxone was 0.14 (0.04–0.46), p = 0.001; and the OR for patients with wound infections was 0.24 (0.09–0.61), p = 0.003. The prevalence of ESBL-producing gram-negative bacteria due to CTX-M in this setting is relatively low compared to other previous reports in similar settings. However, to properly stop further spread in the hospital, we recommend setting up a hospital surveillance system that takes full advantage of the available next-generation sequencing facility to routinely screen for all types of bacterial resistance genes.
Escherichia coli can cause a variety of extra-intestinal infections, such as urinary tract infection, meningitis, peritonitis and septicemia....

General information
State: Accepted/In press
Organisations: Department of Bio and Health Informatics, Genomic Epidemiology, Research Group for Genomic Epidemiology, Statens Serum Institute, University of Washington
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Web of Science (2017): Indexed Yes
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Web of Science (2016): Indexed yes
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BFI (2014): BFI-level 1
Scopus rating (2014): SJR 2.231 SNIP 1.528 CiteScore 3.84
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Web of Science (2013): Indexed yes
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ISI indexed (2012): ISI indexed yes
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Hospital Epidemiology of Methicillin-Resistant Staphylococcus aureus in a Tertiary Care Hospital in Moshi, Tanzania, as Determined by Whole Genome Sequencing

Objective. To determine molecular epidemiology of methicillin-resistant S. aureus in Tanzania using whole genome sequencing. Methods. DNA from 33 Staphylococcus species was recovered from subcultured archived Staphylococcus isolates. Whole genome sequencing was performed on IlluminaMiseq using paired-end 2x250 bp protocol. Raw sequence data were analyzed using online tools. Results. Full susceptibility to vancomycin and chloramphenicol was observed. Thirteen isolates (43.3%) resisted cefoxitin and other antimicrobials tested. Multilocus sequence typing revealed 13 different sequence types among the 30 S. aureus isolates, with ST-8 (n = seven, 23%) being the most common. Gene detection in S. aureus stains were as follows: mecA, 10 (33.3%); pvl, 5 (16.7%); tst, 2 (6.7%). The SNP difference among the six Tanzanian ST-8MRSA isolates ranged from 24 to 196 SNPs and from 16 to 446 SNPs when using the USA300_FPR3757 or the USA500 2395 as a reference, respectively. The mutation rate was 1.38 x 10(-11) SNPs/site/year or 1.4 x 10(-6) SNPs/site/year as estimated by USA300 FPR3757 or the USA500 2395, respectively. Conclusion. S. aureus isolates causing infections in hospitalized patients in Moshi are highly diverse and epidemiologically unrelated. Temporal phylogenetic analysis provided better resolution on transmission and introduction of MRSA and it may be important to include this in future routines.

General information

State: Published

Organisations: National Food Institute, Research Group for Genomic Epidemiology, Department of Bio and Health Informatics, Genomic Epidemiology, KCRI Kilimanjaro Clinical Research Institute, University of Copenhagen

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Identification and Characterization of Variant Intolerant Sites across Human Protein 3-Dimensional Structures

General information
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Organisations: Department of Bio and Health Informatics, Genomic Epidemiology, Broad Institute of Harvard University and Massachusetts Institute of Technology, Massachusetts General Hospital, University of Cologne, University of Luxembourg, Danish Epilepsy Center
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Scopus rating (2014): SJR 2.21 SNIP 1.15 CiteScore 3.33
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 2.245 SNIP 1.156 CiteScore 3.64
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
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Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
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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.
The medical threat of mamba envenoming in sub-Saharan Africa revealed by genus-wide analysis of venom composition, toxicity and antivenomics profiling of available antivenoms

Mambas (genus Dendroaspis) are among the most feared of the medically important elapid snakes found in sub-Saharan Africa, but many facets of their biology, including the diversity of venom composition, remain relatively understudied. Here, we present a reconstruction of mamba phylogeny, alongside genus-wide venom gland transcriptomic and high-resolution top-down venomic analyses. Whereas the green mambas, D. viridis, D. angusticeps, D. j. jamesoni and D. j. kaimosae, express 3FTx-predominant venoms, black mamba (D. polylepis) venom is dominated by dendrotoxins I and K. The divergent terrestrial ecology of D. polylepis compared to the arboreal niche occupied by all other mambas makes it plausible that this major difference in venom composition is due to dietary variation. The pattern of intrageneric venom
variability across Dendroaspis represented a valuable opportunity to investigate, in a genus-wide context, the variant
toxicity of the venom, and the degree of paraspecific cross-reactivity between antivenoms and mamba venoms. To this
end, the immunological profiles of the five mamba venoms were assessed against a panel of commercial antivenoms
generated for the sub-Saharan Africa market. This study provides a genus-wide overview of which available antivenoms
may be more efficacious in neutralising human envenomings caused by mambas, irrespective of the species responsible.
The information gathered in this study lays the foundations for rationalising the notably different potency and
pharmacological profiles of Dendroaspis venoms at locus resolution. This understanding will allow selection and design of
toxin immunogens with a view to generating a safer and more efficacious pan-specific antivenom against any mamba
envenomation.

**General information**

State: Published
Organisations: Network Engineering of Eukaryotic Cell factories, Department of Bio and Health Informatics, Genomic
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BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.03 SNIP 1.051
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ISI indexed (2010): ISI indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 0.785 SNIP 0.949
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Analysis of 62 hybrid assembled human Y chromosomes exposes rapid structural changes and high rates of gene conversion

The human Y-chromosome does not recombine across its male-specific part and is therefore an excellent marker of human migrations. It also plays an important role in male fertility. However, its evolution is difficult to fully understand because of repetitive sequences, inverted repeats and the potentially large role of gene conversion. Here we perform an evolutionary analysis of 62 Y-chromosomes of Danish descent sequenced using a wide range of library insert sizes and high coverage, thus allowing large regions of these chromosomes to be well assembled. These include 17 father-son pairs, which we use to validate variation calling. Using a recent method that can integrate variants based on both mapping and de novo assembly, we genotype 10898 SNVs and 2903 indels (max length of 27241 bp) in our sample and show by father-son concordance and experimental validation that the non-recurrent SNP and indel variation on the Y chromosome tree is called very accurately. This includes variation called in a 0.9 Mb centromeric heterochromatic region, which is by far the most variable in the Y chromosome. Among the variation is also longer sequence-stretches not present in the reference genome but shared with the chimpanzee Y chromosome. We analyzed 2.7 Mb of large inverted repeats (palindromes) for variation patterns among the two palindrome arms and identified 603 mutation and 416 gene conversions events. We find clear evidence for GC-biased gene conversion in the palindromes (and a balancing AT mutation bias), but irrespective of this, also a strong bias towards gene conversion towards the ancestral state, suggesting that palindromic gene conversion may alleviate Muller’s ratchet. Finally, we also find a large number of large-scale gene duplications and deletions in the palindromic regions (at least 24) and find that such events can consist of complex combinations of simultaneous insertions and deletions of long stretches of the Y chromosome.

General information
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Organisations: Department of Bio and Health Informatics, Integrative Systems Biology, Metagenomics, Department of Systems Biology, Center for Biological Sequence Analysis, Integrative Systems Biology, Disease Intelligence and Molecular Evolution, Genomic Epidemiology, Functional Human Variation, Aarhus University, Technical University of Denmark, University of Bergen, Karolinska Institutet, BGI-Europe, University of Bristol, University of Copenhagen, Københavns Universitet, BGI-Shenzhen


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Bacterial whole genome-based phylogeny: construction of a new benchmarking dataset and assessment of some existing methods

Background
Whole genome sequencing (WGS) is increasingly used in diagnostics and surveillance of infectious diseases. A major application for WGS is to use the data for identifying outbreak clusters, and there is therefore a need for methods that can accurately and efficiently infer phylogenies from sequencing reads. In the present study we describe a new dataset that we have created for the purpose of benchmarking such WGS-based methods for epidemiological data, and also present an analysis where we use the data to compare the performance of some current methods.

Results
Our aim was to create a benchmark data set that mimics sequencing data of the sort that might be collected during an
outbreak of an infectious disease. This was achieved by letting an E. coli hypermutator strain grow in the lab for 8 consecutive days, each day splitting the culture in two while also collecting samples for sequencing. The result is a data set consisting of 101 whole genome sequences with known phylogenetic relationship. Among the sequenced samples 51 correspond to internal nodes in the phylogeny because they are ancestral, while the remaining 50 correspond to leaves. We also used the newly created data set to compare three different online available methods that infer phylogenies from whole-genome sequencing reads: NDtree, CSI Phylogeny and REALPHY. One complication when comparing the output of these methods with the known phylogeny is that phylogenetic methods typically build trees where all observed sequences are placed as leaves, even though some of them are in fact ancestral. We therefore devised a method for post processing the inferred trees by collapsing short branches (thus relocating some leaves to internal nodes), and also present two new measures of tree similarity that takes into account the identity of both internal and leaf nodes.

Conclusions

Based on this analysis we find that, among the investigated methods, CSI Phylogeny had the best performance, correctly identifying 73% of all branches in the tree and 71% of all clades. We have made all data from this experiment (raw sequencing reads, consensus whole-genome sequences, as well as descriptions of the known phylogeny in a variety of formats) publicly available, with the hope that other groups may find this data useful for benchmarking and exploring the performance of epidemiological methods. All data is freely available at: https://cge.cbs.dtu.dk/services/evolution_data.php.
Cross-recognition of a pit viper (Crotalinae) polyspecific antivenom explored through high-density peptide microarray epitope mapping

Snakebite antivenom is a 120 years old invention based on polyclonal mixtures of antibodies purified from the blood of hyper-immunized animals. Knowledge on antibody recognition sites (epitopes) on snake venom proteins is limited, but may be used to provide molecular level explanations for antivenom cross-reactivity. In turn, this may help guide antivenom development by elucidating immunological biases in existing antivenoms. In this study, we have identified and characterized linear elements of B-cell epitopes from 870 pit viper venom protein sequences by employing a high-throughput methodology based on custom designed high-density peptide microarrays. By combining data on antibody-peptide interactions with multiple sequence alignments of homologous toxin sequences and protein modelling, we have determined linear elements of antibody binding sites for snake venom metalloproteases (SVMPs), phospholipases A2s (PLA2s), and snake venom serine proteases (SVSPs). The studied antivenom antibodies were found to recognize linear elements in each of the three enzymatic toxin families. In contrast to a similar study of elapid (non-enzymatic) neurotoxins, these enzymatic toxins were generally not recognized at the catalytic active site responsible for toxicity, but instead at other sites, of which some are known for allosteric inhibition or for interaction with the tissue target. Antibody recognition was found to be preserved for several minor variations in the protein sequences, although the antibody-toxin interactions could often be eliminated completely by substitution of a single residue. This finding is likely to have large implications for the cross-reactivity of the antivenom and indicate that multiple different antibodies are likely to be needed for targeting an entire group of toxins in these recognized sites.

General information
State: Published
Organisations: Network Engineering of Eukaryotic Cell factories, Department of Bio and Health Informatics, Genomic Epidemiology, Department of Biotechnology and Biomedicine, Integrative Systems Biology, Universidad de Costa Rica
Design, development and experimental trial of a tailored cytotoxic T-cell vaccine against Porcine Reproductive and Respiratory Syndrome Virus-2

Porcine reproductive and respiratory syndrome virus (PRRSV) is one of the most important threats against the global swine production industry. The virus infects alveolar macrophages that leads to respiratory distress, fever, pneumonia and gives way to secondary respiratory pathogens. Infection of sows in late gestation can lead to late term abortion, early farrowing and birth of litters mixed with living, stillborn and mummified fetuses. Two species of PRRSV exist that are closely related in evolution and disease: PRRSV-1 and PRRSV-2. PRRSV has a positive sense RNA genome of about 15 kb and exhibits a high mutation rate that has led to a high degree of diversity within each species. Highly pathogenic strains evolve occasionally with large impact on animal health and production economy. Since its discovery in the late 1980s, massive efforts have been put in the development of an effective vaccine. Despite this, the most effective commercial vaccines available are only partly capable of protecting against a heterologous challenge. Furthermore, these vaccines are based on modified live virus that at more than one occasion have mutated back to a virulent form and have thus promoted rather than prevented viral spread. PRRSV exhibits a wide range of immunoevasive mechanisms that manipulate multiple branches of the porcine immune system. However, evidence exist that a cell-mediated immune (CMI) response is capable of clearing the virus from the organism, although this response is somewhat delayed. In the present PhD thesis, I describe the development of an innovative vaccine for the induction of a cytotoxic T-lymphocyte response against PRRSV-2. A major part of the project outline was to design a vaccine that would protect beyond genetic drift, why focus has been on identifying and selecting conserved epitopes specific for swine leukocyte antigen class I (SLA-I). Briefly, all naturally occurring 9- and 10-mer peptides derived from 104 highly curated PRRSV-2 whole genome sequences were analyzed for their predicted binding capacities against five SLA-alleles. Two methods for epitope prediction was applied (NetMHCpan and Position Scanning Combinatorial peptide library). The outputs of the two methods were combined and the top 2% best candidates were analyzed using the PopCover algorithm, serving to prioritize the candidates according to conservation and SLA allele coverage. Based on this, 53 peptides were purchased for in vitro verification. This was done using the assays Peptide Affinity Assay and Scintillation Proximity Assay for the determination of peptide-SLA (pSLA) binding affinity and stability, respectively. From these analyses it was decided to proceed with three
of the five SLAs in combination with a total of 33 peptides/epitopes. A Classical swine fever virus (CSFV)-based virus replicon particle (VRP) was selected as vaccine platform. This VRP has the same tropism as CSFV and can thus infect dendritic cells that are the major inducers of a CMI response. On basis of this template VRP, 10 vaccine VRPs were designed for the expression of an inserted polyepitope with subsequent degradation via an uncleavable ubiquitination, thereby leading the epitopes into the MHC-I presentation pathway. One VRP was designed as a negative control and encoded an unrelated epitope, while the remaining nine encoded polyepitopes of different combinations of the 33 PRRSV-2 epitopes. Infectivity of the VRPs and the induced polyepitope expression and degradation was verified using flow cytometry. 718 pigs of matching SLA profiles were vaccinated three times over a 10-week period with the control VRP (N=7) or the PRRSV-VRPs (N=11). After this, all pigs were inoculated with a Danish PRRSV-2 field strain and were euthanized after an additional four weeks. Seroconversion for both VRP and PRRSV was confirmed for all pigs. The induction of a CMI response was monitored using interferon-γ (IFN-γ) enzyme-linked immunospot (ELISPOT) assay pre challenge, but unfortunately did not provide any useful data. The setup was improved and post challenge ELISPOT provided evidence of a VRP-induced CMI. Viral load was measured post challenge in serum, but did not indicate any effects of vaccination. Viral load in lungs did however indicate an effect that was significant in one part of the lungs. Conclusively, the present study provides proof-of-concept that a peptide-specific CMI can be induced by vaccination with VRPs encoding conserved epitopes, along with indications of a protective effect on viral load in lungs. However, several improvements must be made to the concept before it can be subjected to field trials.

**General information**

State: Published
Organisations: National Veterinary Institute, Virology, Adaptive Immunology, Department of Bio and Health Informatics, Genomic Epidemiology
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**Development of a web tool for Escherichia coli subtyping based on fimH alleles: Running title: Development of E. coli fimH sub-typing web-tool**

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 sub-types 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.

**General information**

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Organisations: Department of Bio and Health Informatics, Genomic Epidemiology, Statens Serum Institut, University of Washington
Authors: Roer, L. (Ekstern), Tchesnokova, V. (Ekstern), Allessøe, R. L. (Intern), Muradova, M. (Ekstern), Chattopadhyay, S. (Ekstern), Ahrenfeldt, J. (Intern), Thomsen, M. C. F. (Intern), Lund, O. (Intern), Hansen, F. (Ekstern), Hammerum, A. M. (Ekstern), Sokurenko, E. (Ekstern), Hasman, H. (Ekstern)
Evaluating next-generation sequencing for direct clinical diagnostics in diarrhoeal disease

The accurate microbiological diagnosis of diarrhoea involves numerous laboratory tests and, often, the pathogen is not identified in time to guide clinical management. With next-generation sequencing (NGS) becoming cheaper, it has huge potential in routine diagnostics. The aim of this study was to evaluate the potential of NGS-based diagnostics through direct sequencing of faecal samples. Fifty-eight clinical faecal samples were obtained from patients with diarrhoea as part of the routine diagnostics at Hvidovre University Hospital, Denmark. Ten samples from healthy individuals were also included. DNA was extracted from faecal samples and sequenced on the Illumina MiSeq system. Species distribution was determined with MGmapper and NGS-based diagnostic prediction was performed based on the relative abundance of pathogenic bacteria and Giardia and detection of pathogen-specific virulence genes. NGS-based diagnostic results were compared to conventional findings for 55 of the diarrhoeal samples; 38 conventionally positive for bacterial pathogens, two positive for Giardia, four positive for virus and 11 conventionally negative. The NGS-based approach enabled detection of the same bacterial pathogens as the classical approach in 34 of the 38 conventionally positive bacterial samples and predicted the responsible pathogens in five of the 11 conventionally negative samples. Overall, the NGS-based approach enabled pathogen detection comparable to conventional diagnostics and the approach has potential to be extended for the detection of all pathogens. At present, however, this approach is too expensive and time-consuming for routine diagnostics.

General information
State: Published
Organisations: National Food Institute, Research Group for Genomic Epidemiology, Department of Bio and Health Informatics, Genomic Epidemiology, Hvidovre University Hospital, University of Copenhagen
Authors: Joensen, K. G. (Intern), Engsbro, A. L. Ø. (Ekstern), Lukjancenko, O. (Intern), Kaas, R. S. (Intern), Lund, O. (Intern), Westh, H. (Ekstern), Aarestrup, F. M. (Intern)
Pages: 1325-1338
Publication date: 2017
Main Research Area: Technical/natural sciences

Publication information
Journal: European Journal of Clinical Microbiology & Infectious Diseases
Volume: 36
Issue number: 7
ISSN (Print): 0934-9723
Ratings:
BFI (2018): BFI-level 1
Snakebite envenomings represent a neglected public health issue in many parts of the rural tropical world. Animal-derived antivenoms have existed for more than a hundred years and are effective in neutralizing snake venom toxins when timely administered. However, the low immunogenicity of many small but potent snake venom toxins represents a challenge for obtaining a balanced immune response against the medically relevant components of the venom. Here, we employ high-throughput sequencing of the immunoglobulin (Ig) transcriptome of mice immunized with a three-finger toxin and a phospholipase A2 from the venom of the Central American coral snake, Micrurus nigrocinctus. Although exploratory in nature, our indicate results showed that only low frequencies of mRNA encoding IgG isotypes, the most relevant isotype for therapeutic purposes, were present in splenocytes of five mice immunized with 6 doses of the two types of toxins over...
90 days. Furthermore, analysis of Ig heavy chain transcripts showed that no particular combination of variable (V) and joining (J) gene segments had been selected in the immunization process, as would be expected after a strong humoral immune response to a single antigen. Combined with the titration of toxin-specific antibodies in the sera of immunized mice, these data support the low immunogenicity of three-finger toxins and phospholipases A2 found in *M. nigrocinctus* venoms, and highlight the need for future studies analyzing the complexity of antibody responses to toxins at the molecular level.

**General information**
State: Published
Organisations: Network Engineering of Eukaryotic Cell factories, Department of Biotechnology and Biomedicine, Department of Bio and Health Informatics, Genomic Epidemiology, Juno Therapeutics, Finch Therapeutics, Universidad de Costa Rica
Authors: Laustsen, A. H. (Intern), Engmark, M. (Intern), Clouser, C. (Ekstern), Timberlake, S. (Ekstern), Vigneault, F. (Ekstern), Gutiérrez, J. M. (Ekstern), Lomonte, B. (Ekstern)
Number of pages: 18
Publication date: 2017
Main Research Area: Technical/natural sciences

**FurIOS: a web-based tool for identification of Vibrionaceae species using the fur gene**

Gene based methods for identification of species from the Vibrionaceae family have been developed during the last decades to address the limitations of the commonly used 16S rRNA gene phylogeny. Recently, we found that the ferric-uptake regulator gene (fur) can be used as a single identification marker providing species discrimination, consistent with multi-locus sequencing analyses and whole genome phylogenies. To allow for broader and easy use of this marker, we have developed an online prediction service that allows the identification of Vibrionaceae species based on their fur-sequence. The input is a DNA sequence that can be uploaded on the web service; the output is a table containing the strain identifier, e-value, and percentage of identity for each of the matches with rows colored in green for hits with high probability of being the same species. The service is available on the web at: http://www.cbs.dtu.dk/services/furIOS-1.0/.

The fur-sequences can be derived either from genome sequences or from PCR-amplification of the genomic region encoding the fur gene. We have used 191 strains identified as Vibrionaceae based on 16S rRNA gene sequence to test the PCR method and the web service on a large dataset. We were able to classify 171 of 191 strains at the species level and 20 strains remained unclassified. Furthermore, the fur phylogenetics and subsequent in silico DNA-DNA hybridization demonstrated that two strains (ATCC 33789 and ZS-139) previously identified as *Vibrio splendidus* are more closely
related to V. tasmaniensis and V. cyclitrophicus, respectively. FurIOS is an easy-to-use online service that allows the identification of bacteria from the Vibrionaceae family at the species level using the fur gene as a single marker. Its simplistic design and straightforward pipeline makes it suitable for any research environment, from academia to industry.

**General information**

State: Published
Organisations: Department of Systems Biology, Bacterial Ecophysiology and Biotechnology, Novo Nordisk Foundation Center for Biosustainability, iLoop, Center for Biological Sequence Analysis, Department of Bio and Health Informatics, Genomic Epidemiology, Department of Biotechnology and Biomedicine, Bacterial Ecophysiology and Biotechnology
Authors: Machado, H. (Intern), Cardoso, J. (Intern), Giubergia, S. (Intern), Rapacki, K. (Intern), Gram, L. (Intern)
Number of pages: 8
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**Publication information**

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Ratings:
BFI (2018): BFI-level 1
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BFI (2017): BFI-level 1
Scopus rating (2017): SNIP 0.953 SJR 1.242
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.82 SJR 1.282 SNIP 0.993
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.42 SNIP 0.994 CiteScore 2.93
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.519 SNIP 1.069 CiteScore 2.95
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.571 SNIP 1.179 CiteScore 3.32
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Scopus rating (2012): SJR 1.507 SNIP 1.146 CiteScore 3.38
ISI indexed (2012): ISI indexed yes
Web of Science (2012): Indexed yes
BFI (2011): BFI-level 1
Scopus rating (2011): SJR 1.487 SNIP 1.125 CiteScore 3.4
ISI indexed (2011): ISI indexed yes
Web of Science (2011): Indexed yes
BFI (2010): BFI-level 1
Scopus rating (2010): SJR 1.433 SNIP 1.034
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 1
Scopus rating (2009): SJR 1.474 SNIP 0.964
Web of Science (2009): Indexed yes
BFI (2008): BFI-level 1
Scopus rating (2008): SJR 1.398 SNIP 0.979
Web of Science (2008): Indexed yes
Scopus rating (2007): SJR 1.388 SNIP 0.949
High-density peptide microarray exploration of the antibody response in a rabbit immunized with a neurotoxic venom fraction

Polyvalent snakebite antivenoms derive their therapeutic success from the ability of their antibodies to neutralize venom toxins across multiple snake species. This ability results from a production process involving immunization of large mammals with a broad suite of toxins present in venoms. As a result of immunization with this wide range of toxins, many polyvalent antivenoms have a high degree of cross-reactivity to similar toxins in other snake venoms - a cross-reactivity which cannot easily be deconvoluted. As a proof of concept, we aimed at exploring the opposite scenario by performing a high-throughput evaluation of the extent of cross-reactivity of a polyclonal mixture of antibodies that was raised against only a single snake venom fraction. For this purpose, a venom fraction containing short neurotoxin 1 (SN-1; Uniprot accession number P01416, three-finger toxin (3FTx) family), which is the medically most important toxin from the notorious black mamba (Dendroaspis polylepis), was employed. Following immunization of a rabbit, a specific polyclonal antibody response was confirmed by ELISA and immunodiffusion. Subsequently, these antibodies were investigated by high-density peptide microarray to reveal linear elements of recognized epitopes across 742 3FTxs and 10 dendrotoxins. This exploratory study demonstrates in a single immunized animal that cross-reactivity between toxins of high similarity may be difficult to obtain when immunizing with a single 3FTx containing venom fraction. Additionally, this study explored the influence of employing different lengths of peptides in high-density peptide microarray experiments for identification of toxin epitopes. Using 8-mer, 12-mer, and 15-mer peptides, a single linear epitope element was identified in SN-1 with high precision.
Dendroaspis polylepis, Epitope mapping, Short neurotoxin, Single toxin immunization, Three-finger toxin

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Publication: Research - peer-review › Journal article – Annual report year: 2017

Hospital epidemiology of methicillin-resistant Staphylococcus aureus (MRSA) in a tertiary care hospital in Moshi Tanzania as determined by whole genome sequencing

General information
State: Published
Organisations: National Food Institute, Research Group for Genomic Epidemiology, Department of Bio and Health Informatics, Genomic Epidemiology, Technical University of Denmark
Authors: Kumburu, H. H. (Ekstern), Sonda, T. (Ekstern), Leetcharoenphon, P. (Ekstern), van Zwetselaar, M. (Ekstern), Lukjancenko, O. (Intern), Alifrangis, M. (Ekstern), Lund, O. (Intern), Mmbaga, B. T. (Ekstern), Kibiki, G. (Ekstern), Aarestrup, F. M. (Intern)
Meta-analysis of proportion estimates of extended-spectrum-beta-lactamase-producing Enterobacteriaceae in East Africa hospitals

General information
State: Published
Organisations: Department of Bio and Health Informatics, Genomic Epidemiology, National Food Institute, Research Group for Genomic Epidemiology, KCRI Kilimanjaro Clinical Research Institute, Copenhagen University Hospital
Authors: Sonda, T. (Ekstern), Kumburu, H. (Ekstern), van Zwetselaar, M. (Ekstern), Alifrangis, M. (Ekstern), Lund, O. (Intern), Kibiki, G. (Ekstern), Aarestrup, M. F. F. (Intern)
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Main Research Area: Technical/natural sciences

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BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 2.55 SJR 1.583 SNIP 1.182
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.559 SNIP 1.256 CiteScore 2.4
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.315 SNIP 1.102 CiteScore 2.3
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.599 SNIP 1.173 CiteScore 2.7
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.562 SNIP 1.247 CiteScore 2.82
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.576 SNIP 1.133 CiteScore 2.78
ISI indexed (2011): ISI indexed yes
BFI (2010): BFI-level 2
Scopus rating (2010): SJR 1.525 SNIP 1.263
Web of Science (2010): Indexed yes
BFI (2009): BFI-level 2
Scopus rating (2009): SJR 1.276 SNIP 1.27
BFI (2008): BFI-level 2
Scopus rating (2008): SJR 1.233 SNIP 1.144
Scopus rating (2007): SJR 1.603 SNIP 1.513
Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.399 SNIP 1.384
Scopus rating (2005): SJR 1.224 SNIP 1.295
Scopus rating (2004): SJR 1.276 SNIP 1.246
Scopus rating (2003): SJR 1.211 SNIP 1.468
Scopus rating (2002): SJR 1.153 SNIP 1.128
Scopus rating (2001): SJR 0.873 SNIP 1.082
Scopus rating (2000): SJR 0.897 SNIP 1.073
MGmapper: Reference based mapping and taxonomy annotation of metagenomics sequence reads

An increasing amount of species and gene identification studies rely on the use of next generation sequence analysis of either single isolate or metagenomics samples. Several methods are available to perform taxonomic annotations and a previous metagenomics benchmark study has shown that a vast number of false positive species annotations are a problem unless thresholds or post-processing are applied to differentiate between correct and false annotations.

MGmapper is a package to process raw next generation sequence data and perform reference based sequence assignment, followed by a post-processing analysis to produce reliable taxonomy annotation at species and strain level resolution. An in-vitro bacterial mock community sample comprised of 8 genuses, 11 species and 12 strains was previously used to benchmark metagenomics classification methods. After applying a post-processing filter, we obtained 100% correct taxonomy assignments at species and genus level. A sensitivity and precision at 75% was obtained for strain level annotations. A comparison between MGmapper and Kraken at species level, shows MGmapper assigns taxonomy at species level using 84.8% of the sequence reads, compared to 70.5% for Kraken and both methods identified all species with no false positives. Extensive read count statistics are provided in plain text and excel sheets for both rejected and accepted taxonomy annotations. The use of custom databases is possible for the command-line version of MGmapper, and the complete pipeline is freely available as a bitbucket package (https://bitbucket.org/genomicepidemiology/mgmapper). A web-version (https://cge.cbs.dtu.dk/services/MGmapper) provides the basic functionality for analysis of small fastq datasets.

General information
State: Published
Organisations: Department of Bio and Health Informatics, Metagenomics, National Food Institute, Research Group for Genomic Epidemiology, Genomic Epidemiology, Department of Systems Biology, Center for Biological Sequence Analysis
Authors: Petersen, T. N. (Intern), Lukjancenko, O. (Intern), Thomsen, M. C. F. (Intern), Sperotto, M. M. (Intern), Lund, O. (Intern), Aarestrup, F. M. (Intern), Sicheritz-Pontén, T. (Intern)
Number of pages: 13
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ISSN (Print): 1932-6203
Ratings:
BFI (2018): BFI-level 1
Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): SJR 1.164 SNIP 1.111
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.11 SJR 1.236 SNIP 1.101
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 1.427 SNIP 1.136 CiteScore 3.32
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 1.559 SNIP 1.148 CiteScore 3.54
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 1.772 SNIP 1.153 CiteScore 3.94
ISI indexed (2013): ISI indexed yes
Web of Science (2013): Indexed yes
BFI (2012): BFI-level 1
Modifications of TIGIT expression contribute to CD8 T cell exhaustion in chronic virus infection

General information
State: Published
Organisations: Department of Chemistry, Department of Systems Biology, Center for Biological Sequence Analysis, Department of Bio and Health Informatics, Genomic Epidemiology, Karolinska Institutet, University of Pennsylvania, National Institute of Respiratory Diseases, University of California, San Francisco
Authors: Tauriainen, J. (Ekstern), Scharf, L. (Ekstern), Frederiksen, J. W. (Intern), Naji, A. (Ekstern), Ljunggren, H. (Ekstern), Sonnerborg, A. (Ekstern), Lund, O. (Intern), Reyes-Teran, G. (Ekstern), Hecht, F. M. (Ekstern), Deeks, S. G. (Ekstern), Betts, M. R. (Ekstern), Buggert, M. (Ekstern), Karlsson, A. C. (Ekstern)
Pages: 318
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Main Research Area: Technical/natural sciences

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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): SNIP 0.621 SJR 0.891
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 2.03 SJR 0.979 SNIP 0.644
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 0.933 SNIP 0.679 CiteScore 1.97
Multidrug-resistant Neisseria gonorrhoeae infection with ceftriaxone resistance and intermediate resistance to azithromycin, Denmark, 2017

We describe a multidrug-resistant Neisseria gonorrhoeae infection with ceftriaxone resistance and azithromycin intermediate resistance in a heterosexual man in Denmark, 2017. Whole genome sequencing of the strain GK124 identified MSLT ST1903, NG-MAST ST1614 and all relevant resistance determinants including similar penA resistance mutations previously described in ceftriaxone-resistant gonococcal strains. Although treatment with ceftriaxone 0.5 g plus azithromycin 2 g was successful, increased awareness of spread of gonococcal strains threatening the recommended dual therapy is crucial.

General information
Norwegian patients and retail chicken meat share cephalosporin-resistant Escherichia coli and IncK/blaCMY-2 resistance plasmids

In 2012 and 2014 the Norwegian monitoring programme for antimicrobial resistance in the veterinary and food production sectors (NORM-VET) showed that 124 of a total of 406 samples (31%) of Norwegian retail chicken meat was contaminated with extended-spectrum cephalosporin-resistant Escherichia coli. The aim of this study was to compare selected cephalosporin-resistant E. coli from humans and poultry to determine their genetic relatedness based on whole genome sequencing (WGS). E. coli representing three prevalent cephalosporin-resistant multi-locus sequence types (STs) isolated from poultry (n=17) were selected from the NORM-VET strain collections. All strains carried an IncK plasmid with a blaCMY-2 gene. Clinical E. coli isolates (n=284) with AmpC-mediated resistance were collected at Norwegian microbiology laboratories from 2010 to 2014. PCR screening showed that 29 of the clinical isolates harboured both IncK and blaCMY-2. All IncK/blaCMY-2 positive isolates were analysed by WGS-based bioinformatics tools. Analysis of single nucleotide polymorphisms (SNP) in 2.5 Mbp of shared genome sequences showed close relationship with less than 15 SNP differences between five clinical isolates from urinary tract infections, and the ST38 isolates from poultry. Furthermore, all of the 29 clinical isolates harboured IncK/blaCMY-2 plasmid variants highly similar to the IncK/blaCMY-2 plasmid present in the poultry isolates. Our results provide support for the hypothesis that clonal transfer of cephalosporin-resistant E. coli from chicken meat to humans may occur, and may cause difficult to treat infections. Furthermore, these E. coli can be a source of AmpC resistance plasmids for opportunistic pathogens in the human microbiota.

General information
State: Published
Organisations: Department of Bio and Health Informatics, Genomic Epidemiology, Norwegian Institute of Public Health, Norwegian Veterinary Institute, University Hospital of North Norway, Vestfold Hospital Trust, UiT The Arctic University of Norway, Stavanger University Hospital, Akershus University Hospital, Sørlandet Hospital
Authors: Berg, E. S. (Ekstern), Wester, A. L. (Ekstern), Ahrenfeldt, J. (Intern), Mo, S. S. (Ekstern), Slettemeås, J. S. (Ekstern), Steinbakk, M. (Ekstern), Samuelsen, Ø. (Ekstern), Grude, N. (Ekstern), Simonsen, G. S. (Ekstern), Lehr, I. H. (Ekstern), Jørgensen, S. B. (Ekstern), Tofteland, S. (Ekstern), Lund, O. (Intern), Dahle, U. R. (Ekstern), Sunde, M. (Ekstern)
Publication date: 2017
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Web of Science (2018): Indexed yes
BFI (2017): BFI-level 1
Scopus rating (2017): SNIP 1.854 SJR 2.569
Web of Science (2017): Indexed yes
BFI (2016): BFI-level 1
Scopus rating (2016): CiteScore 3.99 SJR 2.424 SNIP 1.757
Web of Science (2016): Indexed yes
BFI (2015): BFI-level 1
Scopus rating (2015): SJR 2.637 SNIP 1.996 CiteScore 5.11
Web of Science (2015): Indexed yes
BFI (2014): BFI-level 1
Scopus rating (2014): SJR 2.73 SNIP 2.22 CiteScore 5.49
Web of Science (2014): Indexed yes
BFI (2013): BFI-level 1
Scopus rating (2013): SJR 2.306 SNIP 1.852 CiteScore 4.92
ISI indexed (2013): ISI indexed yes
Zoonotic transfer of cephalosporin resistant E. coli, Chicken meat, Plasmid-mediated AmpC transmission, Urinary tract infections

DoIs:
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Source: FindIt
Source-ID: 2350907390
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PATH-01. Identification of Prognostic Variables Based on Molecular Profiling of Long-Term and Short-Term Surviving Glioblastoma Patients

Glioblastoma is a devastating disease and despite extensive treatment, overall survival (OS) for these patients remains poor. Yet, a small proportion of glioblastoma patients present relatively long survival over 3 years, but the underlying molecular background separating these long-term survivors (LTS) from short-term survivors (STS) are still insufficiently understood. The purpose of this study was to identify independent prognostic variables for survival by examining molecular profiles of LTS and STS in a clinically well characterized cohort of glioblastoma patients. The cohort consisted of 93 patients diagnosed with primary glioblastoma and treated with radiation therapy plus concomitant and adjuvant chemotherapy as well as bevacizumab administered in the first-line setting or at time of recurrence. Among these, 14 STS (OS36 months) were identified, which were all confirmed being IDHwt. For all patients, RNA had previously been purified from microdissected tumor tissue of the diagnostic specimen and analyzed for expression levels by a customized NanoString platform. This covered 800 genes related to glioblastoma cancer hallmarks, including regulation of angiogenesis and immune response. When comparing expression of these genes in LTS vs. STS using a Welch’s t-test, 14 candidate genes ended up significant (P

General information
State: Published
Organisations: Department of Biotechnology and Biomedicine, Genomic Epidemiology, Department of Bio and Health Informatics, Rigshospitalet, Danish Cancer Society
Authors: Michaelsen, S. R. (Ekstern), Urup, T. (Ekstern), Olsen, L. R. (Intern), Gillberg, L. (Ekstern), Broholm, H. (Ekstern), Grunnet, K. (Ekstern), Grønbæk, K. (Ekstern), Hamerlik, P. (Ekstern), Lassen, U. (Ekstern), Poulsen, H. S. (Ekstern)
Patterns of infections, aetiological agents, and antimicrobial resistance at a tertiary care hospital in northern Tanzania

Objective
To determine the causative agents of infections and their antimicrobial susceptibility at a tertiary care hospital in Moshi, Tanzania, to guide optimal treatment.

Methods
A total of 590 specimens (stool (56), sputum (122), blood (126) and wound swabs (286)) were collected from 575 patients admitted in the medical and surgical departments. The bacterial species were determined by conventional methods and disk diffusion was used to determine the antimicrobial susceptibility pattern of the bacteria isolates.

Results

A total of 249 (42.2%) specimens were culture-positive yielding a total of 377 isolates. A wide range of bacteria was isolated, the most predominant being Gram-negative bacteria: Proteus spp. (n=48, 12.7%), Escherichia coli (n=44, 11.7%), Pseudomonas spp. (n=40, 10.6%), and Klebsiella spp (n=38, 10.1%). Wound infections were characterised by multiple isolates (n=293, 77.7%), with the most frequent being Proteus spp. (n=44, 15%). Pseudomonas (n=37, 12.6%), Staphylococcus (n=29, 9.9%), and Klebsiella spp. (n=28, 9.6%). All S. aureus tested were resistant to penicillin (n=22, 100%) and susceptible to vancomycin. Significant resistance to cephalosporins such as cefazoline (n=62, 72.9%), ceftriaxone (n=44, 51.8%) and ceftazidime (n=40, 37.4%) was observed in Gram-negative bacteria; as well as resistance to cefoxitin (n=6, 27.3%) in Staphylococcus aureus.

Conclusion

The study has revealed a wide range of causative agents, with an alarming rate of resistance to the commonly used antimicrobial agents. Furthermore, the bacterial spectrum differs from those often observed in high-income countries. This highlights the imperative of regular generation of data on aetiological agents and their antimicrobial susceptibility patterns especially in infectious disease endemic settings. The key steps would be to ensure the diagnostic capacity at a sufficient number of sites and implement structures to routinely exchange, compare, analyse and report data. Sentinel sites (hospitals) across the country (and region) should report on a representative subset of bacterial species and their susceptibility to drugs at least annually. A central organizing body should collate the data and report to relevant national and international stakeholders.

General information

State: Published
Organisations: Department of Bio and Health Informatics, Genomic Epidemiology, National Food Institute, Research Group for Genomic Epidemiology, KCRI Kilimanjaro Clinical Research Institute, University of Copenhagen, Kilimanjaro Christian Medical College
Authors: Kumburu, H. H. (Ekstern), Sonda, T. (Ekstern), Mmbaga, B. T. (Ekstern), Alifrangis, M. (Ekstern), Lund, O. (Intern), Kibiki, G. (Ekstern), Aarestrup, F. M. (Intern)
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BFI (2017): BFI-level 2
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Web of Science (2017): Indexed yes
BFI (2016): BFI-level 2
Scopus rating (2016): CiteScore 2.55 SJR 1.583 SNIP 1.182
BFI (2015): BFI-level 2
Scopus rating (2015): SJR 1.559 SNIP 1.256 CiteScore 2.4
BFI (2014): BFI-level 2
Scopus rating (2014): SJR 1.315 SNIP 1.102 CiteScore 2.3
BFI (2013): BFI-level 2
Scopus rating (2013): SJR 1.599 SNIP 1.173 CiteScore 2.7
ISI indexed (2013): ISI indexed yes
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BFI (2012): BFI-level 2
Scopus rating (2012): SJR 1.562 SNIP 1.247 CiteScore 2.82
ISI indexed (2012): ISI indexed yes
BFI (2011): BFI-level 2
Scopus rating (2011): SJR 1.576 SNIP 1.133 CiteScore 2.78
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Patterns of infections, aetiological agents, and antimicrobial resistance at a tertiary care hospital in northern Tanzania

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Organisations: Department of Bio and Health Informatics, Genomic Epidemiology, National Food Institute, Research Group for Genomic Epidemiology, Technical University of Denmark
Authors: Kumburu, H. H. (Ekstern), Sonda, T. (Ekstern), Mmbaga, B. T. (Ekstern), Alifrangis, M. (Ekstern), Lund, O. (Intern), Kibiki, G. (Ekstern), Aarestrup, F. M. (Intern)
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BFI (2013): BFI-level 2
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Perturbed CD8⁺ T cell TIGIT/CD226/PVR axis despite early initiation of antiretroviral treatment in HIV infected individuals

HIV-specific CD8⁺ T cells demonstrate an exhausted phenotype associated with increased expression of inhibitory receptors, decreased functional capacity, and a skewed transcriptional profile, which are only partially restored by antiretroviral treatment (ART). Expression levels of the inhibitory receptor, T cell immunoglobulin and ITIM domain (TIGIT), the co-stimulatory receptor CD226 and their ligand PVR are altered in viral infections and cancer. However, the extent to which the TIGIT/CD226/PVR-axis is affected by HIV-infection has not been characterized. Here, we report that TIGIT expression increased over time despite early initiation of ART. HIV-specific CD8⁺ T cells were almost exclusively TIGIT⁺, had an inverse expression of the transcription factors T-bet and Eomes and co-expressed PD-1, CD160 and 2B4. HIV-specific TIGIThi cells were negatively correlated with polyfunctionality and displayed a diminished expression of CD226. Furthermore, expression of PVR was increased on CD4⁺ T cells, especially T follicular helper (Tfh) cells, in HIV-infected lymph nodes. These results depict a skewing of the TIGIT/CD226 axis from CD226 co-stimulation towards TIGIT-mediated inhibition of CD8⁺ T cells, despite early ART. These findings highlight the importance of the TIGIT/CD226/PVR axis as an immune checkpoint barrier that could hinder future "cure" strategies requiring potent HIV-specific CD8⁺ T cells.

General information
State: Published
Organisations: Department of Bio and Health Informatics, Genomic Epidemiology, Karolinska University Hospital, University of Pennsylvania, National Institute of Respiratory Diseases, University of California
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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.

General information
State: Published
Organisations: Department of Systems Biology, Center for Biological Sequence Analysis, National Food Institute, Research Group for Genomic Epidemiology, Department of Bio and Health Informatics, Genomic Epidemiology, Statens Seruminstitute
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Web of Science (2009): Indexed yes
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Web of Science (2007): Indexed yes
Scopus rating (2006): SJR 1.771 SNIP 1.437
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Prevalence and risk factors of CTX-M Enterobacteriaceae in hospitalised patients at a tertiary hospital in Kilimanjaro, Tanzania

General information
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Organisations: Department of Bio and Health Informatics, Genomic Epidemiology, National Food Institute, Research Group for Genomic Epidemiology, KCRI Kilimanjaro Clinical Research Institute, University of Copenhagen, Kilimanjaro Christian Medical College
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Web of Science (2013): Indexed yes
Recombinant snakebite antivenoms: A cost-competitive solution to a neglected tropical disease?

Snakebite envenoming is a major public health burden in tropical parts of the developing world. In sub-Saharan Africa, neglect has led to a scarcity of antivenoms threatening the lives and limbs of snakebite victims. Technological advances within antivenom are warranted, but should be evaluated not only on their possible therapeutic impact, but also on their cost-competitiveness. Recombinant antivenoms based on oligoclonal mixtures of human IgG antibodies produced by CHO cell cultivation may be the key to obtaining better snakebite envenoming therapies. Based on industry data, the cost of treatment for a snakebite envenoming with a recombinant antivenom is estimated to be in the range USD 60-250 for the Final Drug Product. One of the effective antivenoms (SAIMR Snake Polyvalent Antivenom from the South African Vaccine Producers) currently on the market has been reported to have a wholesale price of USD 640 per treatment for an average snakebite. Recombinant antivenoms may therefore in the future be a cost-competitive alternative to existing serum-based antivenoms.
RUCS: Rapid identification of PCR primers for unique core sequences

Designing PCR primers to target a specific selection of whole genome sequenced strains can be a long, arduous, and sometimes impractical task. Such tasks would benefit greatly from an automated tool to both identify unique targets, and to validate the vast number of potential primer pairs for the targets in silico. Here we present RUCS, a program that will find PCR primer pairs and probes for the unique core sequences of a positive genome dataset complement to a negative genome dataset. The resulting primer pairs and probes are in addition to simple selection also validated through a complex in silico PCR simulation. We compared our method, which identifies the unique core sequences, against an existing tool called ssGeneFinder, and found that our method was 6.5-20 times more sensitive. We used RUCS to design primer pairs that would target a set of genomes known to contain the mcr-1 colistin resistance gene. Three of the predicted pairs were chosen for experimental validation using PCR and gel electrophoresis. All three pairs successfully produced an amplicon with the target length for the samples containing mcr-1 and no amplification products were produced for the negative samples. The novel methods presented in this manuscript can reduce the time needed to identify target sequences, and provide a quick virtual PCR validation to eliminate time wasted on ambiguously binding primers. Source code is freely available on https://bitbucket.org/genomicepidemiology/rucs. Web service is freely available on https://cge.cbs.dtu.dk/services/RUCS. mctf@cbs.dtu.dk. Supplementary data is available at Bioinformatics online.
Hundreds of thousands of human genomes are now being sequenced to characterize genetic variation and use this information to augment association mapping studies of complex disorders and other phenotypic traits. Genetic variation is identified mainly by mapping short reads to the reference genome or by performing local assembly. However, these approaches are biased against discovery of structural variants and variation in the more complex parts of the genome. Hence, large-scale de novo assembly is needed. Here we show that it is possible to construct excellent de novo assemblies from high-coverage sequencing with mate-pair libraries extending up to 20 kilobases. We report de novo assemblies of 150 individuals (50 trios) from the GenomeDenmark project. The quality of these assemblies is similar to those obtained using the more expensive long-read technology. We use the assemblies to identify a rich set of structural variants including many novel insertions and demonstrate how this variant catalogue enables further deciphering of known
association mapping signals. We leverage the assemblies to provide 100 completely resolved major histocompatibility complex haplotypes and to resolve major parts of the Y chromosome. Our study provides a regional reference genome that we expect will improve the power of future association mapping studies and hence pave the way for precision medicine initiatives, which now are being launched in many countries including Denmark.

**General information**

**State:** Published

**Organisations:** Department of Bio and Health Informatics, Metagenomics, Department of Systems Biology, Center for Biological Sequence Analysis, Integrative Systems Biology, Integrative Systems Biology, Disease Intelligence and Molecular Evolution, Genomic Epidemiology, High Performance Computing, Functional Human Variation, University of Copenhagen, Aarhus University, BGI-Shenzhen, BGI-Europe, Technical University of Denmark, University of Oslo, University of Bergen, Karolinska Institutet


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**Main Research Area:** Technical/natural sciences
TANTIGEN: a comprehensive database of tumor T cell antigens

Tumor T cell antigens are both diagnostically and therapeutically valuable molecules. A large number of new peptides are examined as potential tumor epitopes each year, yet there is no infrastructure for storing and accessing the results of these experiments. We have retroactively cataloged more than 1000 tumor peptides from 368 different proteins, and implemented a web-accessible infrastructure for storing and accessing these experimental results. All peptides in TANTIGEN are labeled as one of the four categories: (1) peptides measured in vitro to bind the HLA, but not reported to elicit either in vivo or in vitro T cell response, (2) peptides found to bind the HLA and to elicit an in vitro T cell response, (3) peptides shown to elicit in vivo tumor rejection, and (4) peptides processed and naturally presented as defined by physical detection. In addition to T cell response, we also annotate peptides that are naturally processed HLA binders, e.g., peptides eluted from HLA in mass spectrometry studies. TANTIGEN provides a rich data resource for tumor-associated epitope and neoepitope discovery studies and is freely available at (mirror).

General information
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Organisations: Department of Bio and Health Informatics, Genomic Epidemiology, Dana-Farber Cancer Institute
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The CGE Tool Box

As whole genome sequence data of microorganisms are becoming easily accessible and cheap to produce, a transformation of the traditional methods used for typing, phenotyping and phylogenetic analysis of microorganisms is on the way. Following the anticipation that most clinical microbiological and food safety laboratories will soon have a sequencer in use on a daily basis, there is a growing need for easy-to-use bioinformatics methods that can quickly convert the sequence data into useful information on, e.g., the type of bacteria, whether it is resistant towards any types of antibiotics, and whether it is part of an outbreak. The Center for Genomic Epidemiology, which is located at the Technical University of Denmark, has since its beginning in 2010 developed such bioinformatics methods and made them freely available as web-services. These web-services and their use is the focus of this chapter.

General information
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Transcriptional changes induced by bevacizumab combination therapy in responding and non-responding recurrent glioblastoma patients

Background: Bevacizumab combined with chemotherapy produces clinical durable response in 25-30% of recurrent glioblastoma patients. This group of patients has shown improved survival and quality of life. The aim of this study was to investigate changes in gene expression associated with response and resistance to bevacizumab combination therapy.

Methods: Recurrent glioblastoma patients who had biomarker-accessible tumor tissue surgically removed both before bevacizumab treatment and at time of progression were included. Patients were grouped into responders (n = 7) and non-responders (n = 14). Gene expression profiling of formalin-fixed paraffin-embedded tumor tissue was performed using RNA-sequencing.

Results: By comparing pretreatment samples of responders with those of non-responders no significant difference was observed. In a paired comparison analysis of pre- and posttreatment samples of non-responders 1 gene was significantly differentially expressed. In responders, this approach revealed 256 significantly differentially expressed genes (72 down- and 184 up-regulated genes at the time of progression). Genes differentially expressed in responders revealed a shift towards a more proneural and less mesenchymal phenotype at the time of progression.

Conclusions: Bevacizumab combination treatment demonstrated a significant impact on the transcriptional changes in responders; but only minimal changes in non-responders. This suggests that non-responding glioblastomas progress chaotically without following distinct gene expression changes while responding tumors adaptively respond or progress by means of the same transcriptional changes. In conclusion, we hypothesize that the identified gene expression changes of responding tumors are associated to bevacizumab response or resistance mechanisms.
WGS-based surveillance of third-generation cephalosporin-resistant Escherichia coli from bloodstream infections in Denmark

To evaluate a genome-based surveillance of all Danish third-generation cephalosporin-resistant Escherichia coli (3GC-R Ec) from bloodstream infections between 2014 and 2015, focusing on horizontally transferable resistance mechanisms. A collection of 552 3GC-R Ec isolates were whole-genome sequenced and characterized by using the batch uploader from the Center for Genomic Epidemiology (CGE) and automatically analysed using the CGE tools according to resistance
profile, MLST, serotype and fimH subtype. Additionally, the phylogenetic relationship of the isolates was analysed by SNP analysis. The majority of the 552 isolates were ESBL producers (89%), with bla CTX-M-15 being the most prevalent (50%) gene, followed by bla CTX-M-14 (14%), bla CTX-M-27 (11%) and bla CTX-M-101 (5%). ST131 was detected in 50% of the E. coli isolates, with the remaining isolates belonging to 73 other STs, including globally disseminated STs (e.g. ST10, ST38, ST58, ST69 and ST410). Five of the bloodstream isolates were carbapenemase producers, carrying bla OXA-181 (3) and bla OXA-48 (2). Phylogenetic analysis revealed 15 possible national outbreaks during the 2 year period, one caused by a novel ST131/bla CTX-M-101 clone, here observed for the first time in Denmark. Additionally, the analysis revealed three individual cases with possible persistence of closely related clones collected more than 13 months apart. Continuous WGS-based national surveillance of 3GC-R Ec, in combination with more detailed epidemiological information, can improve the ability to follow the population dynamics of 3GC-R Ec, thus allowing for the detection of potential outbreaks and the effects of changing treatment regimens in the future.
Benchmarking of methods for identification of antimicrobial resistance genes in bacterial whole genome data

Next generation sequencing (NGS) may be an alternative to phenotypic susceptibility testing for surveillance and clinical diagnosis. However, current bioinformatics methods may be associated with false positives and negatives. In this study, a novel mapping method was developed and benchmarked to two different methods in current use for identification of antibiotic resistance genes in bacterial WGS data. A novel method, KmerResistance, which examines the co-occurrence of k-mers between the WGS data and a database of resistance genes, was developed. The performance of this method was compared with two previously described methods; ResFinder and SRST2, which use an assembly/BLAST method and BWA, respectively, using two datasets with a total of 339 isolates, covering five species, originating from the Oxford University Hospitals NHS Trust and Danish pig farms. The predicted resistance was compared with the observed phenotypes for all isolates. To challenge further the sensitivity of the in silico methods, the datasets were also down-sampled to 1% of the reads and reanalysed. The best results were obtained by identification of resistance genes by mapping directly against the raw reads. This indicates that information might be lost during assembly. KmerResistance performed significantly better than the other methods, when data were contaminated or only contained few sequence reads. Read mapping is superior to assembly-based methods and the new KmerResistance seemingly outperforms currently available methods particularly when including datasets with few reads.

General information
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Organisations: Department of Bio and Health Informatics, National Food Institute, Genomic Epidemiology, Research Group for Genomic Epidemiology, Department of Systems Biology, Center for Biological Sequence Analysis
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Number of pages: 5
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Main Research Area: Technical/natural sciences

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Volume: 71
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Biotechnological Trends in Spider and Scorpion Antivenom Development

Spiders and scorpions are notorious for their fearful dispositions and their ability to inject venom into prey and predators, causing symptoms such as necrosis, paralysis, and excruciating pain. Information on venom composition and the toxins present in these species is growing due to an interest in using bioactive toxins from spiders and scorpions for drug discovery purposes and for solving crystal structures of membrane-embedded receptors. Additionally, the identification and isolation of a myriad of spider and scorpion toxins has allowed research within next generation antivenoms to progress at an increasingly faster pace. In this review, the current knowledge of spider and scorpion venoms is presented, followed by a discussion of all published biotechnological efforts within development of spider and scorpion antitoxins based on small molecules, antibodies and fragments thereof, and next generation immunization strategies. The increasing number of discovery and development efforts within this field may point towards an upcoming transition from serum-based antivenoms towards therapeutic solutions based on modern biotechnology.
Multi-omic profiling of EPO producing Chinese hamster ovary cell panel reveals metabolic adaptation to heterologous protein production

Heterologous protein production in CHO cells imposes a burden on the host cell metabolism and impact cellular physiology on a global scale. In this work, a multi-omics approach was applied to characterize the physiological impact of erythropoietin production, and discover production bottlenecks, in a panel of CHO-K1 cells in batch and chemostat culture.

General information
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Organisations: Novo Nordisk Foundation Center for Biosustainability, CHO Cell Line Engineering and Design, Quantitative Modeling of Cell Metabolism, Network Engineering of Eukaryotic Cell factories, Department of Bio and Health Informatics, Genomic Epidemiology, Department of Biotechnology and Biomedicine, Novo Nordisk A/S
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VAR2CSA Epitope Identification – Finding All the Needles in All the Haystacks

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Organisations: Department of Bio and Health Informatics, National Veterinary Institute, Immunoinformatics and Machine Learning, T-cells & Cancer, Genomic Epidemiology, University of Copenhagen, Schafer-N, Copenhagen University Hospital
Authors: Jessen, L. E. (Intern), Ditlev, S. B. (Ekstern), Schafer-Nielsen, C. (Ekstern), Buus, S. (Ekstern), Salanti, A. (Ekstern), Lund, O. (Intern)
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Scopus rating (2013): SJR 0.875 SNIP 0.709 CiteScore 2.05
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BFI (2010): BFI-level 1
Scopus rating (2010): SJR 0.859 SNIP 0.621
BFI (2009): BFI-level 1
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Web of Science (2009): Indexed yes
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Project participant:
Christensen, Julia (Intern)
Project