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Publications:

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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)
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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
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Organisations: National Food Institute, Research Group for Genomic Epidemiology, Department of Bio and Health Informatics, Genomic Epidemiology, Hvidovre University Hospital, University of Copenhagen
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Audouin's gull, a potential vehicle of an extended spectrum beta-lactamase producing Salmonella Agona

The genome of a multidrug-resistant Salmonella Agona isolated from Larus audouinii (Audouin's gull) in Spain was examined. The isolate showed high levels of resistance to different antimicrobials, including third generation cephalosporins and fluoroquinolones, which is a public health concern as those being used to treat severe salmonellosis in humans. Whole genome sequencing revealed the strain being multilocus sequence type ST13, and eight resistance genes (aadA2, aadB, bla(CTX-M-9), bla(DHA-1), qnrA1, tetA, sul1 and dfrA16) belonging to seven antimicrobial classes were confirmed, as well as the presence of two plasmids. Migratory Audouin's gulls have the ability to cover long distances during annual movements. Therefore, they have the potential to disseminate multidrug-resistant Salmonella and resistance genes in the environment and over great geographic distances, contributing to the global dissemination of resistance genes.

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Rapid and Easy In Silico Serotyping of Escherichia coli Isolates by Use of Whole-Genome Sequencing Data

Accurate and rapid typing of pathogens is essential for effective surveillance and outbreak detection. Conventional serotyping of Escherichia coli is a delicate, laborious, time-consuming, and expensive procedure. With whole-genome sequencing (WGS) becoming cheaper, it has vast potential in routine typing and surveillance. The aim of this study was to establish a valid and publicly available tool for WGS-based in silico serotyping of E. coli applicable for routine typing and surveillance. A FASTA database of specific O-antigen processing system genes for O typing and flagellin genes for H typing was created as a component of the publicly available Web tools hosted by the Center for Genomic Epidemiology (CGE) (www.genomicepidemiology.org). All E. coli isolates available with WGS data and conventional serotype information were subjected to WGS-based serotyping employing this specific SerotypeFinder CGE tool. SerotypeFinder was evaluated on 682 E. coli genomes, 108 of which were sequenced for this study, where both the whole genome and the serotype were available. In total, 601 and 509 isolates were included for O and H typing, respectively. The O-antigen genes wzx, wzy, wzm, and wzt and the flagellin genes flIC, flkA, flIA, flmA, and flnA were detected in 569 and 508 genome sequences, respectively. SerotypeFinder for WGS-based O and H typing predicted 560 of 569 O types and 504 of 508 H types, consistent with conventional serotyping. In combination with other available WGS typing tools, E. coli serotyping can be performed solely from WGS data, providing faster and cheaper typing than current routine procedures and making WGS typing a superior alternative to conventional typing strategies.

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What Can We Learn from a Metagenomic Analysis of a Georgian Bacteriophage Cocktail?

Phage therapy, a practice widespread in Eastern Europe, has untapped potential in the combat against antibiotic-resistant bacterial infections. However, technology transfer to Western medicine is proving challenging. Bioinformatics analysis could help to facilitate this endeavor. In the present study, the Intesti phage cocktail, a key commercial product of the Eliava Institute, Georgia, has been tested on a selection of bacterial strains, sequenced as a metagenomic sample, de novo assembled and analyzed by bioinformatics methods. Furthermore, eight bacterial host strains were infected with the cocktail and the resulting lysates sequenced and compared to the unamplified cocktail. The analysis identified 23 major phage clusters in different abundances in the cocktail, among those clusters related to the ICTV genera T4likevirus, T5likevirus, T7likevirus, Chilikevirus and Twortlikevirus, as well as a cluster that was quite distant to the database sequences and a novel Proteus phage cluster. Examination of the depth of coverage showed the clusters to have different abundances within the cocktail. The cocktail was found to be composed primarily of Myoviridae (35%) and Siphoviridae (32%), with Podoviridae being a minority (15%). No undesirable genes were found.

Applying the ResFinder and VirulenceFinder web-services for easy identification of acquired antibiotic resistance and E. coli virulence genes in bacteriophage and prophage nucleotide sequences.

Extensive research is currently being conducted on the use of bacteriophages for applications in human medicine, agriculture and food manufacturing. However, phages are important vehicles of horizontal gene transfer and play a significant role in bacterial evolution. As a result, concern has been raised that this increased use and dissemination of phages could result in spread of deleterious genes, e.g., antibiotic resistance and virulence genes. Meanwhile, in the wake of the genomic era, several tools have been developed for characterization of bacterial genomes. Here we describe how
two of these tools, ResFinder and VirulenceFinder, can be used to identify acquired antibiotic resistance and virulence genes in phage genomes of interest. The general applicability of the tools is demonstrated on data sets of 1,642 phage genomes and 1,442 predicted prophages.

**Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic Escherichia coli.**

Fast and accurate identification and typing of pathogens are essential for effective surveillance and outbreak detection. The current routine procedure is based on a variety of techniques, making the procedure laborious, time-consuming, and expensive. With whole-genome sequencing (WGS) becoming cheaper, it has huge potential in both diagnostics and routine surveillance. The aim of this study was to perform a real-time evaluation of WGS for routine typing and surveillance of verocytotoxin-producing Escherichia coli (VTEC). In Denmark, the Statens Serum Institut (SSI) routinely receives all suspected VTEC isolates. During a 7-week period in the fall of 2012, all incoming isolates were concurrently subjected to WGS using IonTorrent PGM. Real-time bioinformatics analysis was performed using web-tools (www.genomicepidemiology.org) for species determination, multilocus sequence type (MLST) typing, and determination of phylogenetic relationship, and a specific VirulenceFinder for detection of E. coli virulence genes was developed as part of this study. In total, 46 suspected VTEC isolates were characterized in parallel during the study. VirulenceFinder proved successful in detecting virulence genes included in routine typing, explicitly verocytotoxin 1 (vtx1), verocytotoxin 2 (vtx2), and intimin (eae), and also detected additional virulence genes. VirulenceFinder is also a robust method for assigning verocytotoxin (vtx) subtypes. A real-time clustering of isolates in agreement with the epidemiology was established from WGS, enabling discrimination between sporadic and outbreak isolates. Overall, WGS typing produced results faster and at a lower cost than the current routine. Therefore, WGS typing is a superior alternative to conventional typing strategies. This approach may also be applied to typing and surveillance of other pathogens.
Two cases of extremely drug-resistant Salmonella enterica serovar Senftenberg isolated from patients in Zambia were investigated by utilizing MIC determinations and whole-genome sequencing. The isolates were resistant to, and harbored genes toward, nine drug classes, including fluoroquinolones and extended-spectrum cephalosporins, contained two plasmid replicons, and differed by 93 single-nucleotide polymorphisms.

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Real-Time WGS-based Typing of VTEC Isolates for Surveillance and Outbreak Detection

Objectives: Fast and accurate typing of foodborne pathogens is essential for effective surveillance and the ability to detect and prevent outbreaks. Current routine typing is based on a variety of different typing techniques, making the complete typing procedure laborious, time-consuming and expensive. With whole-genome sequencing (WGS) becoming continuously cheaper and more available, it has huge potential in both diagnostics and routine surveillance. The aim of this study was to evaluate WGS-based typing, in a real-time setup, for routine typing and surveillance of verocytotoxin-producing E.coli (VTEC) infections.

Methods: As part of the routine surveillance in Denmark, suspected VTEC isolates are sent to Statens Serum Institut (SSI) for phenotypic and molecular characterisation by a range of methods. During 7 weeks in the fall 2012, the isolates were simultaneously subjected to WGS using the IonTorrent PGM benchtop sequencing technology. WGS-based typing was carried out using web-based tools, developed by the Center for Genomic Epidemiology (www.genomicepidemiology.org), for determination of MLST types, virulence genes and phylogenetic relationship between the isolates. The WGS-based typing was compared to the routine typing and surveillance, with regard to typing results, time consumption and price.

Results: In total, 47 suspected VTEC isolates were typed during the 7 weeks, both by the routine procedures and in parallel by the WGS-approach, and during the period of the study a small outbreak occurred. For all isolates, apart from one resulting in poor sequence output, the WGS-based typing led to detection of the same virulence gene variants as the routine typing, and was also able to detect many other possible virulence features, and in most instances produce a useful typing result faster than routine typing. Also, the WGS-approach was able to correctly detect, according to the routine typing, the isolates belonging to the outbreak.

Conclusion: The real-time WGS-based typing was able to produce typing results comparable to the routine typing, at least as fast as the routine typing. Thus, the benchtop WGS-based typing approach is a reasonable alternative to conventional typing strategies, and could be applicable to typing and surveillance of other pathogens.

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Hasman, Henrik (Intern)  
Main Supervisor:  
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Persson, Søren (Ekstern)  

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