Detection and Molecular Epidemiology of Foodborne and Animal Pathogens

IZSLER Parma laboratory adds the MiSeq® System to perform pathogen testing, epidemiology, and genetic screening studies efficiently.

Introduction

The city of Parma is located in north central Italy's beautiful Po River valley. The fertile lower valley is often referred to as "food valley", and is home to several gastronomic delights, including Prosciutto di Parma ham, Parmigiano-Reggiano cheese, as well as several international food companies. It's no wonder that Parma is the seat of the European Food Safety Authority (EFSA) and a laboratory of the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia Romagna (IZSLER) that is committed to animal health and food safety.

As the veterinarian in charge of risk assessment at IZSLER and Head of IZSLER Parma, Stefano Pongolini, DVM, oversees a laboratory team of 20 researchers and laboratory technologists, focusing on the study, diagnosis, and prophylaxis of animal diseases and food microbiology. With the recent addition of next-generation sequencing (NGS) to their analysis tool set, IZSLER Parma is performing viral and bacterial testing more efficiently, and providing services to a larger customer population.

iCommunity spoke with Dr. Pongolini about the IZSLER Parma laboratory, the testing services it provides, and how the MiSeq System is enabling them to track foodborne illnesses and pathogens effectively.

Q: What types of services does IZSLER Parma provide to agri-food businesses and how does it support public health?
Stefano Pongolini (SP): IZSLER is an official Institute of Veterinary Public Health and operates at a regional level within the National Health Service of Italy. We provide diagnostic and testing services for 2 economically important regions, Lombardy and Emilia-Romagna, which represent about 23% of the Italian population and 30% of the GNP. Our routine testing activities serve the Regional Health Authorities and local businesses. More research-oriented activities, such as the studies we conduct using NGS, are offered to a broader group of clients within and outside the region.

Our services can be grouped into 5 main areas: 1) animal disease diagnostic testing, including zoonoses; 2) official controls on food and feedstuffs; 3) epidemiological and analytical support to health authorities for the design and management of official prevention and eradication plans; 4) monitoring and surveillance of animal and zoonotic diseases for foodborne diseases; and 5) applied and basic research in infectious diseases.

IZSLER Parma is also the home of 2 reference centers. The Regional ENTER-NET Reference Center runs the Enteric-Pathogens (foodborne) surveillance system of the Emilia-Romagna region, covering a total population of about 5,000,000 individuals. This surveillance includes tracking human cases of foodborne diseases. The World Organization for Animal Health (OIE) is also represented here with the Reference Laboratory for Swine Influenza.

Q: What types of pathogen testing do you perform?
SP: There are many viruses and bacteria that we test for at the center, some that cause animal disease, and others that contribute to foodborne human illness and disease. We offer testing for several swine viruses, including swine influenza virus, and porcine reproductive and respiratory syndrome virus. We also can screen for various bovine viruses, including bovine viral diarrhea virus, bovine syncytial virus, bovine rotavirus and coronavirus, and bovine rhinotracheitis virus. In addition, we test for Hepatitis A virus and were able to contribute to the tracking of a 2013 HAV outbreak in Europe.[1] We also test for flaviviruses, which include West Nile and the Usutu virus (USUV) that have recently been on the rise in Europe.

The diagnosis and surveillance of bacterial pathogens encompasses the full spectrum of animal and foodborne pathogens. These include *Escherichia coli*, as well as bacteria in the *Salmonella*, *Klebsiella*, *Clostridium*, *Listeria*, *Staphylococcus*, *Streptococcus*, *Pseudomonas*, *Pasteurella*, *Actinobacillus*, and *Mycoplasma* genera. Because this is an important milk and cheese producing region, we're involved in the diagnosis of bovine mastitis, testing over 20,000 milk samples annually for bacteria associated with the infection.

Dr. Stefano Pongolini is the veterinarian in charge of risk assessment at IZSLER and Head of IZSLER Parma.

Q: Ten years ago, what technologies did you use for food pathogen detection and typing?
SP: Over the last decade, the diagnostic techniques we've used for pathogen detection have shifted from simple agar-based differential/selective media and biochemical tests to PCR-based molecular methods. The standard microbiology techniques are still used widely as initial diagnostics, but these methods don't provide the data necessary to perform pathogen typing for epidemiological investigations. They also don't deliver the amount of information needed to depict the distribution of pathogens in the target population (animal or human) and along the food chain.

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Q: What made you consider adding NGS tools at your IZSLER Parma laboratory?
SP: With NGS, we can access all the genetic variant information that a pathogen stores in its chromosome as it's spreading in the environment from an intermediate host or food to humans. This type of information is pivotal in supporting an epidemiological investigation. We can also use NGS to identify the sources of infection and routes of transmission, along with virulence and antimicrobial resistance genes. The greatest advantage of NGS is that it enables us to use a single method to obtain all this data, rather than performing multiple analyses using several time-intensive techniques such as pulse-field gel electrophoresis (PFGE), multiple-locus variable number tandem repeat analysis (MLVA), multilocus sequence typing (MLST), or antibiograms.

Q: Why did you choose the MiSeq System over other NGS systems?
SP: We chose the MiSeq System because of its base calling quality, the absence of homopolymer bias, and the high read length and yield it can achieve at an affordable instrument cost.

Q: What types of sequencing do you perform with the MiSeq System?
SP: We have been using the MiSeq System to perform whole-genome analyses of bacterial and viral isolates whenever a foodborne outbreak is suspected or detected within our regional surveillance mandate. The speed and multiplexing capabilities of the MiSeq System enable us to perform epidemiology and genetic screening studies efficiently.

Q: What epidemiology studies have you performed with the MiSeq System?
SP: When we first got the MiSeq System, we wanted to implement our pipelines and evaluate the potential of this new genomic tool. So we used the MiSeq System to analyze retrospectively a human outbreak of infections by *Salmonella enterica* serovar Manhattan, a rare serovar in humans that occurred in Italy in 2009. In particular, we performed a differential single nucleotide polymorphism (SNP) analysis in comparison with the gold-standard genotyping method, PFGE.[2] In that study, a total of 39 isolates were analyzed from patients, food, feed, animal, and environmental sources, resulting in 5 different PFGE profiles. Isolates epidemiologically related to the outbreak clustered within the same pulsotype, SXB_BS.0003, without any further differentiation. Upon reanalysis by whole-genome sequencing based on total core SNPs, 4 distinct groups of isolates emerged within the outbreak pulsotype. Interestingly, outbreak-related human isolates where distinct from food-origin isolates associated with the outbreak. Following detailed analysis based on different SNP subsets, the same distinction was shown by nonsynonymous SNPs, as well as SNPs at the second and first plus second codon positions. Conversely, analyses derived from synonymous and third codon position SNPs that are less sensitive to selective pressure, showed that the food and human isolates clustered together. This indicated that all outbreak-related isolates constituted a single clone, which was in line with the epidemiological evidence. This work proved to us that subclonal genotyping based on differences in even a few SNPs (5–6) is a powerful tool for our diagnostic routine. It also highlighted the importance of carefully evaluating the output of bioinformatic analyses and the data sets used. The study was an encouraging milestone, taking advantage of the high fidelity of base calling, and therefore SNP identification, of the MiSeq System.

Q: How long did it take to add the MiSeq System into your workflow?
SP: We adopted the MiSeq System in late October 2013 and included it in our workflow (library preparation and running samples) within 1 month of installation. From a practical point of view, we were up and running easily and obtained workable results for epidemiological applications quickly. Preparation of sequencing libraries is the most sensitive step of the process and it took some fine-tuning to obtain stable results. We found that it's important to spend the time up front in evaluating the quality of DNA input and setting up multiplexing protocols to ensure precise and accurate data generation.

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Q: How many sequencing runs do you perform each week?
SP: We usually perform 1–2, 2 x 250 paired-end runs per week. We use the Nextera® XT Library Preparation Kit for creating sequencing-ready libraries of bacterial and viral DNA.

Sample number varies depending on the genome size of the pathogens. Considering a mean genome of 4.5 Mbp, we multiplex 10 bacteria plus up to 30 viruses per run.

Q: Why did you choose the Nextera XT Library Preparation Kit?
SP: We're working with bacterial and viral clinical isolates and often deal with extremely small samples. We chose the Nextera XT Library Prep Kit because it enables us to prepare high-quality libraries from just 1 ng of input DNA.

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Q: What data analysis software do you use?
SP: Our data analysis pipelines are based almost exclusively on open-source software, which we use to perform sequencing reads QC, draft genome assembly and annotation, variant detection, and phylogenetic analysis of clinical isolates.

Q: Do you include sequencing data in the reports you provide to customers?
SP: We are a public health laboratory, so our main NGS activities are focused on epidemiological surveillance of human and animal pathogens within the horizon of official surveillance plans. From this perspective, we include the relevant details of our data analyses, including sequencing data, in the technical reports sent to the competent authorities.

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Q: How do you see NGS enhancing or expanding your service offering in the future?
SP: Molecular epidemiology relies on precise typing of clinical and environmental isolates to solve important problems in public health, especially for outbreak investigations. The more variants, mainly SNPs, that we can describe for each single strain under scrutiny, the higher the probability of finding the sources of infections and routes of transmission, along with virulence and fitness determinants of pathogens.

In the past, this information was obtained using several different analytical methods, each one having limitations and lengthy workflow times that could jeopardize the fast response of health authorities to an infectious outbreak. The MiSeq System has proven it can provide all this information from a single run quickly and efficiently. It takes just 3 days from clinical isolates in pure culture to analyzed sequence data.

In addition, the MiSeq System can access all the information stored in a pathogen's genome, providing the data to infer correct pathogen typing. In fact, the amount of informative genetic data the MiSeq System provides is greater than the sum of the data generated using all 'traditional' methods, enabling the identification of correct isolate relatedness even without epidemiological metadata. With NGS costs rapidly decreasing and data analysis pipelines being tested and validated worldwide, we look forward to replacing all our traditional analytical methods with a single, dedicated NGS pipeline.

References

1. Fitzgerald M, Thornton L, O'Gorman J, et al. Outbreak of a hepatitis A infection associated with the consumption of frozen berries, Ireland, 2013—linked to an international outbreak. *Euro Surveill.* 2014; 19(43) pii: 20942.
2. Scaltriti E, Sassera D, Comandatore F, et al. Differential single nucleotide polymorphism-based analysis of an outbreak caused by *Salmonella enterica* serovar Manhattan reveals epidemiological details missed by standard pulsed-field gel electrophoresis. *J Clin Microbiol.* 2015; 53:1227–1238.

Learn more about the Illumina product and system mentioned in this article:

- MiSeq System, www.illumina.com/systems/miseq.html
- Nextera XT DNA Library Preparation Kit, www.illumina.com/products/nextera_xt_dna_library_prep_kit.html

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