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The Importance of Establishing a Whole-Genome Sequencing Network and Database for Food-Pathogen Tracing

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ABSTRACT:

The Food and Drug Administration (FDA) has established a system of state, federal, international, and commercial partners in the United States for open-source whole-genome sequencing. When it comes to identifying and tracking down the origins of infections that cause foodborne outbreaks, the GenomeTrakr network is the first of its type. GenomeTrakr is at the forefront of food poisoning outbreak research and compliance action, allowing for more precise and prompt recalls of tainted foods and better monitoring of preventative controls in food processing facilities. To aid in a timely public health response to bacterial outbreaks, an increased network's capacity for pathogen traceback rapid monitoring is essential.

INTRODUCTION:

Food-borne illness continues to be a major worldwide issue to public health, as seen by recent deadly outbreaks linked to the eating of freshly cut fruit. About 1 in 6 Americans, or 48 million individuals, become sick from contaminated food each year, leading to 128,000 hospitalizations and 3,000 deaths (http://www.cdc.gov/foodborneburden). According to the Produce Safety Project (http://www.pewtrusts.org/en/about/news-room/press-releases/0001/01/01 /foodborne-illness-costs-nation-$152-billion-annually-nearly -$39-billion-loss-attributed-to-produce), the annual cost of foodborne illness is estimated to be $152 billion. It might seem like an insurmountable task to reduce the prevalence of food-borne disease.

The inability to quickly determine the origin of food contamination is a long-standing issue. Previous technology, pulsed-field gel electro- phoresis (PFGE), despite the efforts of food safety specialists, typically lacked the precision needed to properly locate the source of an epidemic. In a retrospective outbreak analysis of a 2012 Salmonella epidemic related to spicy tuna sushi rolls using PFGE, researchers at the U.S. Food and Drug Administration's Center for Food Safety and Applied Nutrition (FDA-CFSAN) saw the potential of whole-genome se- quencing (WGS). All of the PFGE-matching clinical, food, and historical isolates were sequenced on an Illumina MiSeq. Although PFGE showed no discernible difference between current epidemic isolates and unrelated history isolates, WGS revealed an unexpectedly high degree of resolution separating all of the isolates. The epidemic's isolates were most closely related to a 5-year-old historical iso- late that was traced to a processing factory about 8 kilometers from the epicenter of the outbreak (1). This isolate, like many others, was stored in the FDA-CFSAN's freezer collection after being discovered during a prior check of tainted seafood at the port of entry. The FDA-CF- SAN's decision to invest more broadly in WGS technology (http://www.fda.gov/Food/FoodScienceResearch/WholeGenomeSequencingProgramWGS/) was motivated by the possibility of sequencing all of the FDA's historical isolates, which would provide investigators with geographic clues from a large high-resolution genomic database. In order to realize this goal, the FDA- CFSAN established a pilot network of federal and state labs (4

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state, and 9 FDA regional laboratories). This decentralized network, known as the FDA Food Emergency Response Network (FERN) GenomeTrakr (GT) (https://www.youtube.com/watch?v=EsrHu6ozsz8), began collecting WGS data from pathogenic bacteria in food in 2012 and promptly uploading it to a publicly accessible database managed by the National Center for Biotechnology Information (NCBI) at the National Institutes of Health (http://www.ncbi.nlm.nih. The NCBI, together with the EMBL in Europe and the DDBJ in Asia, make up the International Nucleotide Sequence Database Collaboration (Fig. 1). The three DNA databases share their data nightly, making the combined resource truly worldwide. Over 30 laboratories throughout the United States and abroad are now connected via GenomeTrakr (GT), with several of the state labs also participating in the FDA Food Emergency Response Network (FERN). One important feature of this network is the widespread dissemination of draft genomes, which allows for the rapid identification of developing genetic clusters or matches and the timely provision of information to assist ongoing investigations (http://www.ncbi.nlm.nih.gov/pathogens/). Our aim is to build and harmonize databases on a national and worldwide scale to improve the quality and reach of this network.

Currently, the network (Fig. 1) consists of 30 separate laboratories, many of which are sponsored by the FDA-CFSAN and have access to dedicated personnel as well as Illumina MiSeq desktop sequencers, supporting equipment, and consumables/reagents. The FDA-CFSAN also manages a web-based communication platform for disseminating important papers and hosting troubleshooting forums to assist with the network's laboratory and bioinformatics requirements. The FDA-CFSAN now receives the vast majority of sequences directly from individual MiSeqs and qual-

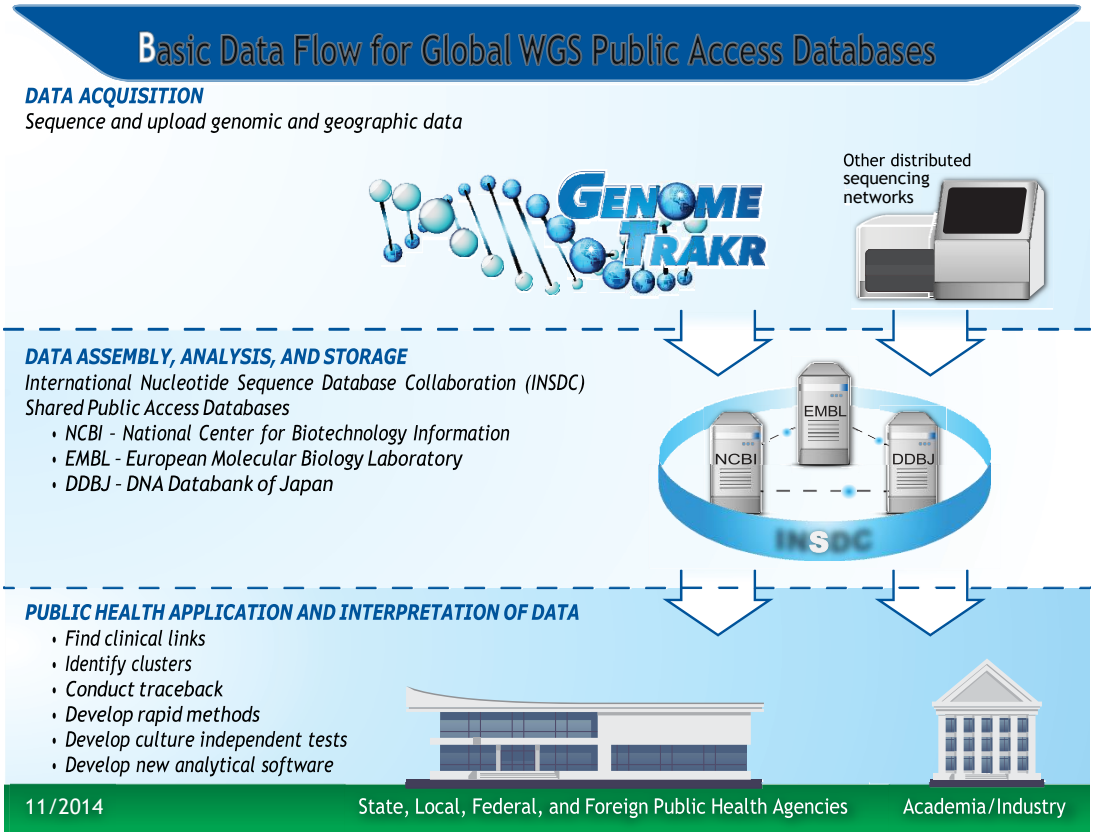

FIG 1 Data flow for the GenomeTrakr database and network.

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ity checked and formatted for upload to the NCBI GenomeTrakr database. The FDA-CFSAN is working with the NCBI and com- mercial software vendors (Illumina and Qiagen CLC bio as well as others) to develop and release tools that will allow individual lab- oratories to add sequences to the public databases directly and independently. It is important to note that this project receives strong in-kind support from the NCBI, and a description of their tools and software will be reported elsewhere (see http://www.ncbi .nlm.nih.gov/pathogens/).

There are two keys to the success of the GenomeTrakr for im- proving food safety. One is the creation of a centralized, globally accessible database comprising a widely diverse set of pathogen genomes that was collected from known locations and food types. As the reference database grows, the likelihood that new sequences "match" something in the database increases, which provides clues and context to the new sample and increases our knowledge on the root causes of foodborne contamination. The "open data" part of this is a huge leap forward from the PFGE database model, which is restricted to a set of public heath agencies. This open model will increase the diversity of the database by encouraging contribution from academic, industry, and international part- ners. In addition, these same partners now have access to data that are identical to those the public health agencies are using for out- break detection and trackback. This approach provides useful and timely data to the public. The second key is the "rapid uploading" aspect of the GenomeTrakr data collection and sharing. Newly sequenced draft genomes from foodborne pathogens collected from clinical patients, facilities, and food are all rapidly shared directly after data collection.

This enables effective monitoring of foodborne pathogens across the United States and potentially across the globe. Our discussions so far have focused on genomic data—the As, Ts, Gs, and Cs comprising each genome. However, these data are only as valuable as their associated metadata. To ensure the usefulness of the metadata, GenomeTrakr imple- mented a minimum set of metadata fields, required for all food and environmental isolates. These fields include the following general who, what, when, and where information: who collected the isolate, its taxonomic name, date of collection (to day, month, or year), county of origin, U.S. state, and isolation source (e.g., cilantro, avocado, environmental swab). This information is enough for coarse tracking, but it is not fine enough to implicate any one facility or farm. More detailed metadata information, such as specific geographic location and brand names, are kept private and confidential. The quality of the metadata connected to a draft genome greatly expands the utility of the sequence, en- abling the FDA and other partners to track the origin of pathogen reservoirs and to discover specific geographic regions that may harbor unique pathogen types. Releasing minimum metadata in real time allows linkages to be made across global sampling, con- tributing to the discovery of connections that may assist in an investigation or support root cause to improve preventative con-trols. Any reasons made for delaying the full release of the draft genomic data or metadata have the potential to delay the discovery of those connections to the detriment of public health. For this reason, the GenomeTrakr network is committed to public release of data and metadata in real time. It is notable that other national

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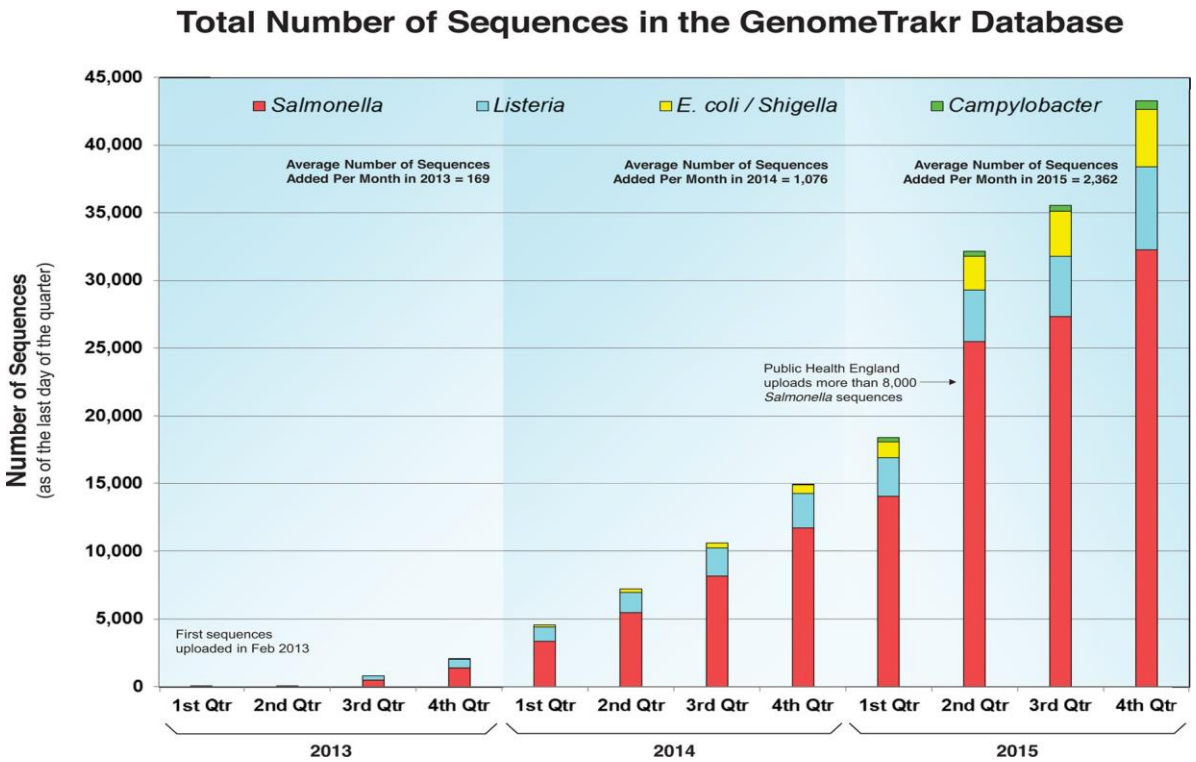

and international public health agencies that are currently in- volved in WGS-based surveillance legitimately have different con- cepts about the level of metadata that should be publically acces- sible. For example, the CDC delays the release of clinical isolate metadata for 6 months; however, once updated, the fields them- selves are harmonized.

Although GenomeTrakr was initially conceived for outbreak source tracking, the database allows the FDA to gather other cru- cial information, including (i) antimicrobial resistance (AMR) (http://www.ridom.com/company/; http://arpcard.mcmaster.ca/; https://github.com/iqbal-lab/Mykrobe-predictor), (ii) serological characterization without the need for classical antibody testing (http://www.denglab.info/SeqSero), and (iii) virulence pathogenic- ity assessment for emerging bacterial or viral pathogens (http://www .cbs.dtu.dk/~dhany/reads2type.html; http://www.genomicepidemi- ology.org/; http://www.mgc.ac.cn/cgi-bin/VFs/genus.cgi?Genus =Escherichia; https://cge.cbs.dtu.dk/services/VirulenceFinder/) (2– 13). Ultimately, our goal is to facilitate the distribution of sequencing capability to as many sites as possible so that public health laborato- ries can move sequences from their freezer collections and current sampling workload into the database as quickly as possible to support ongoing investigations and improve public health.

The current GenomeTrakr database contains roughly 33,000 *Salmonella* isolates, 7,000 *Listeria* isolates, 5,000 *E. coli*/*Shigella* isolates, and 1,000 *Campylobacter* isolates. It is growing roughly 1,000 new draft genomes per month or 1 every hour (Fig. 2). Daily phylogenetic trees showing emerging linkages and relatedness are generated by the NCBI and are publicly accessible (see http://www .ncbi.nlm.nih.gov/pathogens/).

Regulatory offices at the FDA are using the WGS data and daily phylogenetic trees to identify new contamination events, which are being uncovered on a daily/ weekly basis. As the database expands, this high-resolution tool will continue to provide new insights into outbreak causes and risks as well as the compliance of past contaminators.

VISION

An expanded GenomeTrakr network, covering the entire country with 50 or more public health laboratories, would act as a com-prehensive food shield for the United States by providing a robust system for sentinel surveillance and pathogen traceback. This rep- resents a key technological advancement for microbiological out- break investigations, enabling public health agencies to greatly improve their ability to track the microbiological contamination of foods to their sources. The use of GenomeTrakr data (genome plus metadata) has already assisted public health officials in pin- pointing the sources responsible for multiple foodborne out- breaks faster than traditional methods (14). Continual expansion of the database will also benefit the food industry in several differ- ent ways. An enhanced capability of food producers to self-regu- late will allow them tighter control over the finished product and the safety of raw material supply lines. Integration of WGS into the private sector will generate new products and services in the form of software and easy-to-use tools for incorporating genomic data into food safety plans. Expansion of the WGS network would most optimally occur with national and international oversight to en-sure that the database remains fully accessible and populated with minimal metadata to serve as a valuable resource to clinicians, scientists, regulators, and the business community.

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RATIONALE AND SIGNIFICANCE

Expanded deployment of WGS technology to additional state lab- oratories will allow for the collection of thousands of important new enteric pathogen sequences. It gets equipment closer to the hands of the first responders. State laboratories have already con- tributed to the four GenomeTrakr pathogen databases, providing diverse genomic data for foodborne outbreak characterization and traceback. Moreover, as the technology helps pinpoint previ- ously unknown sources of contamination, this knowledge will be used to update good agricultural and manufacturing practices. Based on new WGS revelations, the FDA is designing targeted guidance to help manufacturers avoid future pathogen contami- nation along the farm-to-fork continuum. The health and eco- nomic impacts that are gained by using a WGS-based surveillance system may be significant. For example, a comparison of nut but- ter outbreaks from pre-WGS and post-WGS technology reveals huge public health consequences. In a 2014 event, after the FDA had adopted the use of WGS to assist in outbreak investigations, nut butter from a single manufacturer was contaminated with *Salmonella*, causing 5 people to report food poisoning. Compari- son of this event to a similar event in 2009, prior to the FDA's adoption of WGS technology, was striking. In the earlier outbreak, 9 people died and at least 714 people in 46 states, half of them children, fell ill due to food poisoning from eating products con- taining peanuts. Among persons with available information, an astounding 23% reported being hospitalized. During the 2014 event, in which WGS sentinel surveillance was available, early in- tervention by regulatory authorities, significantly fewer illnesses, and only one hospitalization were reported. WGS data were brought to bear very early during the 2014 investigation, and this likely prevented significant illness, hospitalizations, and future in- cidence of chronic sequelae associated with foodborne Salmonel- losis, including Reiter's syndrome and Guillain-Barre syndrome. It is difficult to predict the actual impact of early intervention, but it is clear that the earlier contaminated food is identified and con- tained, the fewer people will be affected by the contamination event. Newly funded WGS laboratories would only need to solve one or two events per year to provided added value to these hidden costs to public health.

The initial growth of the GenomeTrakr project leveraged ex-
isting partnerships with other federal and state agencies and forged new ones with academia and international labs. The FDA worked with the NCBI to standardize the input data for Genome-Trakr and to build tools for analyzing and viewing the results. The FDA also partnered closely with the Centers for Disease Control and Prevention and the U.S. Department of Agriculture's Food Safety Inspection Service to include isolates from clinical and meat samples in the database. The FDA partnered with state public health labs, who were willing to collaborate and had collections of historical pathogen isolates in their freezers. Finally, the FDA worked with the FDA's Center for Veterinary Medicine (CVM), a component of the National Antimicrobial Monitoring System (NARMS), to sequence over 1,000 foodborne NARMS isolates with known antimicrobial resistances. This allowed for direct comparison of genetic and phenotypic measures of AMR. Initial estimates of the correlation between genetic and phenotypic markers are very high, which indicates that WGS is a viable ap- proach for early identification of emerging AMR profiles and their host pathogens (4–7) just through monitoring the existing data-bases.

PROPOSED WGS EXPANSION

Recent examples show the unprecedented power and utility of the GenomeTrakr model, which lends support to continued expan-sion of this model. The GenomeTrakr project began in the fall of 2012 with 13 laboratories and expanded to 15 in 2013, 19 in 2014,and 30 in 2015. In the fall of 2013, the CDC and partners (FDA, USDA, NCBI, and a few state public health labs) proposed a col-laborative sequencing effort for *Listeria monocytogenes*. In this project, every *L. monocytogenes* isolate collected in the United States would be sequenced and analyzed within 1 week of isolation (14). In March of 2014, the FDA, using its new powers provided by the Food Safety Modernization Act (FSMA), closed a contami- nated cheese facility in Delaware that had sickened people in mul- tiple states. This decision was made by incorporating WGS data from clinical samples and food and environmental swabs collected during inspection of the food facility. WGS of *Listeria monocyto-genes* strains isolated from cheese products and from the facility in Delaware was performed by the FDA and Virginia's Division of Consolidated Laboratory Services. These strains were found to be highly related by WGS to the *Listeria* strains isolated from patients in this outbreak, adding further confidence that cheese products produced by company X were a likely source of the outbreak.Compared with pulsed-field gel electrophoresis (PFGE), WGSwas able to provide a clearer distinction between cases and foods that are likely part of a given outbreak and those that are not. This was the first time WGS was used in a regulatory action by the FDA. Company X has voluntarily recalled all of their cheese products distributed in Delaware, Maryland, New Jersey, New York, Vir- ginia, and the District of Columbia. The FDA suspended the food facility registration of company X of Delaware on 11 March 2014 after it was determined that there was a reasonable probability of food manufactured, processed, packed, or held by company X causing serious adverse health consequences or death to humans. To our knowledge, this was the first use of WGS for a federal regulatory action, and it documented the successful coordination of deploying WGS across the federal agencies of HHS and the USDA Food Safety and Inspection Service (FSIS) and state partner integration. In the summer of 2014, GenomeTrakr labs detected a *Salmonella* contamination event in nut butter across several states with low levels of contamination in a widely distributed product.In this case, WGS identified the link and preempted an outbreakeven without the availability of an isolate from a specific food but rather only with the pathogen collected from contaminated equipment at the food facility uncovered during an office of com-pliance inspection.

This WGS evidence thus informed the epide- miology, and our inspectors with the CDC confirmed the link between food and clinical samples. These proven methods for the early identification of pathogens and rapid response will greatly reduce the public health burden of outbreaks that often go on for months and get many people ill.

In the summer of 2014, the FDA conducted environmental sampling at almond and peanut butter facilities as part of an as- signment designed to gather baseline data on the presence of food-borne pathogens in nut butter-processing facilities. Samples from one of the facilities tested positive for *Salmonella enterica* serovar Braenderup. PFGE and WGS analyses were performed. The PFGE patterns were indistinguishable from several additional isolates, so whole-genome sequencing was performed on these isolates as well and was compared to those from the environmental samples to determine their relatedness. The pathogens from the few samples that were available were an extremely close match. In this instance, WGS achieved multiple foodborne illness investigation tasks, in- cluding confirming that clinical isolates were related to each other and to the nut butter and showing that the strain that caused the illnesses was identical to the strain of *Salmonella* Braenderup iso- lated from the processing facility. This is significant because the high degree of certainty in determining the relatedness of patho- gens may provide important traceback investigation clues, even though, to that point in time, traditional epidemiology methods had not revealed a common food consumed by the individuals who had become ill. By utilizing WGS early in the process to de-finitively characterize pathogens, the causes of sporadic food- borne illnesses may become known even before they cause a pub- lic health crisis. Early intervention also benefited the food industry that was responsible, as understanding the root cause and source of the outbreak allowed the company to clean their facility and resume production of safe nut butters.

The nut butter example also documents an important compo- nent of WGS evidence, which is that sometimes WGS leads the epidemiology and sometimes it is the other way around. Isolates that are more rapidly placed into the public domain allow for this kind of cold hit discovery to take place, which allows for the dis- covery of low-level contamination events and previously hidden pathogen sources that may be affecting public health. Currently, all positive pathogen isolates from FDA inspections are routinely sequenced and uploaded to the WGS database to look for any linkage between food, environment, and clinical isolates. The FDA acts on promising WGS investigational leads through their regu- latory tools and procedures. WGS evidence is never used in isola- tion, and there is always independent support from inspection, including positive culture and or epidemiological concordance, before any regulatory actions are implemented.

GENOMETRAKR PROPOSED EXPANSION

The FDA's priority is to expand the WGS network capacity in foods and to equip more state health and agriculture laboratories so that the investigators who are inspecting foods and collecting pathogens from food and the environment can sequence these newly acquired isolates as they are discovered. Currently, the tech- nology is too expensive for most state laboratories to adopt inde- pendently of new funds, but with initial startup funds, we have already seen several of the state GenomeTrakr laboratories (New York and Minnesota) successfully identify new sources of patho- gens (15, 16). An expansion of this and other networks will expo- nentially increase the number of outbreaks discovered, with more known samples being populated into the database as well as more actions arising from inspections. The advanced understanding of where pathogens reside will improve preventative controls so that food industries produce safer foods. Rather than a new draft ge- nome per hour, additional state labs would increase this to a new pathogen characterized every 15 min, with daily linkages discov- ered between pathogens from patients, food, and the environ- ment. This expanded capacity will also allow more databases of other critical pathogen species to be created. Several states (e.g., New York) are already expanding their portfolio of pathogens beyond foodborne pathogens to include other regional needs such as TB, West Nile virus, and other infectious pathogens. For less than $1,500,000, a large database (n > 5,000) can be constructed for any targeted pathogen species. Many of the states in the exist- ing network belong to the Food Emergency Response Network (FERN; www.fernlab.org), which would play a significant role in a national emergency related to the food supply. The costs for build- ing the existing GenomeTrakr network have been borne largely by the FDA-CFSAN. The current GenomeTrakr network has numer- ous state, federal, international, and commercial contract labora- tories actively uploading data, with many new laboratories plan- ning to collaborate. For a recent list of contributing labs, see http://www.fda.gov/Food/FoodScienceResearch/WholeGenome SequencingProgramWGS/ucm363134.htm. There are three basic costs for implementing the expansion of a WGS network to addi- tional states: initial equipment as a one-time cost, annual costs including reagents and salaries for technicians to run the sequenc- ers, and instrument maintenance costs. The NCBI bears the costs for data storage, quality checks, access, preliminary phylogenetic analyses, and characterization tools.

OUTREACH AND HARMONIZATION EFFORTS

The FDA-CFSAN is expanding outreach to industry, which per- forms the vast majority of food safety monitoring compared to the public sector. Genomics is a new field that some industry leaders (IBM, Mars, DuPont, Nestle, General Mills, and ConAgra, to name a few) are beginning to implement in their own food safety monitoring efforts. There are many applications in the area of food quality and standardization that would immediately benefit from the

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use of these genomic technologies. Food manufacturers could use the highly discriminatory data provided by WGS to track the source of pathogen contaminations to a supplier of in-gredients or to a specific environmental niche in the manufactur-ing environment. The data could be used to allow manufacturers to efficiently detect and correct problems, which is consistent with most modern food safety system concepts (good manufacturing practices [GMPs] and hazard analysis and critical control point [HACCP]) as well as with the requirements of the FDA's recently implemented FSMA. In addition, the availability of WGS from industry isolates, like isolates from raw ingredients, could allow outbreaks to be detected much earlier, resulting in much lower case counts and economic damage resulting from lawsuits and harm to brand reputation. The degree to which the food industry adopts this new technology depends not only on the cost of ac-quiring the technology but also on the potential costs that a man-ufacturer would be implicated as the cause of foodborne illness. Food industry outreach and education will be conducted through coordination with various food associations, such as the Institute for Food Safety and Health (IFSH). The IFSH is a longtime partner of FDA-CFSAN s Moffet Center and will engage the industry in wet laboratory and bioinformatics training. The FDA also engages agriculture extension services for outreach directly to growers through university affiliates.

Global outreach will include coordination with organizations such as the World Health Organization (WHO), the United Na- tions Food Agricultural Organization (FAO), and the Interna- tional Standards Organization (ISO). This includes efforts in training as well as validation, harmonization, and integration across the global community. The World Health Organization (WHO) has several groups interested in the role of WGS and advancement in global public health, including the WHO Global Foodborne Infections Network (WHO-GFN) and the WHO In- ternational Food Standards, CODEX Committee on Food Hy- giene. The United Nations Food Agricultural Organization is also meeting, harmonizing, and discussing global efforts to build ca- pacity to detect, control, and prevent foodborne and other enteric infections in close association with HHS and the USDA. The key objective of these agencies is to enhance laboratory-based surveil- lance of foodborne disease worldwide by improving laboratory capacity for microbiological identification, characterization, and epidemiological investigations. These international bodies seek to facilitate communication through a One Health perspective, mak- ing connections between public health, agricultural, and veteri-nary sectors about relevant pathogens and food safety hazards, which is a critical step toward standardized global epidemiology of infectious diseases. One Health espouses the idea of fully connect- ing the clinical, food, and farm environments as an integrated whole rather than as disconnected parts.

WGS is revolutionizing the characterization of infectious dis- ease diagnoses as well as public health surveillance and response. In addition, the ability to share sequence data is leading to major changes in global health monitoring, especially once international agreements are reached on standardized databases for reporting and analyzing such sequence data and when numerous legal and ethical solutions are sorted out. In foodborne disease detection and response, the application of whole-genome sequencing con- stitutes a major leap forward in technology for local and global public health laboratories. It allows a single test to replace several less efficient and more expensive technologies that require special-ized training. For example, it is possible to extract serotype infor- mation from a bacterial pathogen genome along with antibiotic resistance patterns and the relatedness of strains in an outbreak(2–13). This will obviate the need to support multiple methodol-ogies for the various pathogenic species. The expectation is that WGS will contribute to a broad-reaching mitigation of the burden of infectious disease globally through the understanding of each strains relationship with those from other ill patients in the com-munity, from food, and from the environment.

The One Health approach combined with WGS and phylogenetics will help un- cover root cause data for infection and preventative controls for lasting solutions.

The WHO-FAO and their expert panels and white papers will provide guidance to countries to help determine when to invest in the technology, what needs to be in place before WGS can add value to a public health system, and how to utilize WGS for prac- tical applications for public health purposes. The WHO-FAO rep- resents a well-established network of laboratories and institutions that are dedicated to building laboratory capacity. In the case of WGS, the WHO-FAO can facilitate global knowledge transfer rap-idly through their standing committees. This potential expanded role is so promising, and the consequences of leaving developing countries behind are so dramatic that the WHO-FAO has already begun to lead discussions on WGS (e.g., recently CODEX in Bos- ton, 2015, and FAO-WHO Expert Meeting on practical applica- tions in Rome, 2015). Relying on WHO recommendations, the FDA-CFSAN placed a WGS instrument in Argentina in 2015 to pilot the capacity in the Pan American Health Organization (PAHO) region. The ultimate goal is to broadly disseminate WGS capacity to other regions where appropriate.

EXPANSION OF NETWORK TO CLINICAL LABORATORIES

While the clinic and other nonfood-related components are be-yond FDA-CFSAN s regulatory scope or jurisdiction, expansion of WGS networks to clinical laboratories would provide a more comprehensive genomic shield to address infectious disease threats of public health importance. To gain maximum surveil- lance of the pathogens circulating and impacting public health, the FDA recommends taking the One Health approach. What the existing consortia has not fully realized is connecting WGS tech- nology to the clinical hospital environment or establishing a broad network of global partners that upload data from their geographic regions. From a broader perspective, it is important to note that such a distributed genomic "digital immune system" (D. Lipman, personal communication, 17) could serve to link illnesses that are associated with numerous bacterial or viral agents of significance to public health and to national security. Such a deployment of genomic resources may significantly contribute to an early warn- ing system, including sentinel surveillance for endemic or epi- demic

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characterization of nosocomial and community-acquired strains. One integrated system would also provide links for mon-itoring antimicrobial resistance and the detection of virulence de- terminants among more aggressive infectious agents. Hospitals represent the first responders to epidemic and zoonotic infectious agents, as they emerge in the population. To this end, we are closely watching pilot clinical work flows that permit hospitals tofeed WGS data through to the NCBI pipeline for direct upload and curation (18–21). Laboratories like these are successfully using WGS data to identify common resistance genes and for the pur- poses of evaluating transmissibility and are part of clinical labora- tory networks working to integrate WGS data and a controlled level of associated metadata with a public interface and open- source curation. We believe that early successes associated with the clinic can be expanded at the national and global levels to develop an early warning system for AMR monitoring and other clinical sectors of the microbial disease community.

CONCLUSION

WGS methods represent an unprecedented new approach for tracking pathogens. Here, we document the potential for food- borne pathogen surveillance networks on an international scaleand suggest that the approach taken by the FDA-CFSAN and the FERN GenomeTrakr network can be emulated and expanded to include clinical and global partner laboratories. In parallel with the NCBI's efforts to establish an international database and re- pository for these data, now is the time to evaluate the utility of a broader interconnected system for detection and typing of out-break strains at the international level in the form of a compre- hensive network of sequencers. WGS is here for the benefit of public health, and we are now challenged with the next step for- ward in network expansion. The FDA-CFSAN s top priority is for direct application in foods to provide invaluable source tracking information for important outbreak pathogens. It is time to con- sider building a larger integrated WGS Network and connecting it to other international ones like it for an expanded role as national public and private health care assets.

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REFERENCES

1. **Hoffmann M, Luo Y, Monday SR, Gonzales-Escalona N, Ottensen**
2. **A, Muruvanda T, Wang C, Kastanis G, Keys C, Janies D, Senturk I, Catalyurek UV, Wang H, Hammack TS, Wolfgang WJ,**
3. **Schoonmaker- Bopp D, Chu A, Myers R, Haendiges J, Evans P, Meng J, Strain E, Allard MW, Brown EW.** 2016. Tracing origins of the *Salmonella* Bareilly strain causing a food-borne outbreak in the United States. J Infect Dis **213:**502–508. http://dx.doi.org/10.1093/infdis/jiv297.
4. **Hoffmann M, Zhao S, Pettengill J, Luo Y, Monday SR, Abbott J, AyersSL, Cinar HN, Muruvanda T, Li C, Allard M, Whichard J, Meng J, Brown EW, McDermott PF.** 2014. Comparative genomic analysis and virulence differences in closely related *Salmonella enterica* serotype Heidelberg isolates from humans, retail meats, and animals. Genome Biol Evol **6:**1046 –1068. http://dx.doi.org/10.1093/gbe/evu079.
5. **Chen Y, Mukherjee S, Hoffmann M, Kotewicz M, Young S, Abbott J, Luo Y, Davidson M, Allard MW, McDermott PF, Zhao S.** 2013. Whole- genome sequencing of gentamicin-resistant *Campylobacter coli* isolated from U.S. retail meats reveals novel plasmid mediated aminoglycoside resistance genes. Antimicrob Agents Chemother **57:**5398 –5405. http://dx .doi.org/10.1128/AAC.00669-13.
6. **Zhao S, Tyson GH, Chen Y, Li C, Mukherjee S, Young S, Lam C, Folster JP, Whichard JM, McDermott PF.** 2015. Whole-genome sequencing analysis accurately predicts antimicrobial resistance phenotypes in *Cam-pylobacter* spp. Appl Environ Microbiol **82:**459 – 466. http://dx.doi.org/10 .1128/AEM.02873-15.
7. **Gordon NC, Price JR, Cole K, Everitt R, Morgan M, Finney J, Kearns AM, Pichon B, Young B, Wilson DJ, Llewelyn MJ, Paul J, Peto TE, Crook DW, Walker AS, Golubchik T.** 2014. Prediction of *Staphylococcus aureus* antimicrobial resistance by whole-genome sequencing. J Clin Mi- crobiol **52:**1182–1191. http://dx.doi.org/10.1128/JCM.03117-13.
8. **Stoesser N, Batty EM, Eyre DW, Morgan M, Wyllie DH, Del Ojo Elias C, Johnson JR, Walker AS, Peto TE, Crook DW.** 2013. Predicting antimicrobial susceptibilities for *Escherichia coli* and *Klebsiella pneu- moniae* isolates using whole genomic sequence data. J Antimicrob Che-mother **68:**2234 –2244.
9. **Zankari E, Hasman H, Kaas RS, Seyfarth AM, Agerso Y, Lund O, Larsen MV, Aarestrup FM.** 2013. Genotyping using whole- genome se- quencing is a realistic alternative to surveillance based on phenotypic an- timicrobial susceptibility testing. J Antimicrob Chemother **68:**771–777. http://dx.doi.org/10.1093/jac/dks496.
10. **Cao G, Allard MW, Hoffmann M, Monday SR, Muruvanda T, Luo Y, Payne J, Rump L, Meng K, Zhao S, McDermott PF, Brown EW, Meng J.** 2015. Complete sequences of six IncA/C plasmids of multidrug-resistant *Salmonella enterica* subsp. *enterica* serotype Newport. Genome Announc **3:**e00027–15. http://dx.doi.org/10.1128/genomeA.00027-15.
11. **Carraro N, Matteau D, Burrus V, Rodrigue S.** 2015. Unraveling the regulatory network of IncA/C plasmid mobilization: When genomic is- lands hijack conjugative elements. Mobile Genetic Elements **5:**1–5. http: //dx.doi.org/10.1080/2159256X.2015.1006109.
12. **McArthur AG, Waglechner N, Nizam F, Yan A, Azad MA, Baylay AJ, Bhullar K, Canova MJ, De Pascale G, Ejim L, Kalan L, King AM, Koteva K, Morar M, Mulvey MR, O'Brien JS, Pawlowski AC, Piddock VLJ, Spanogiannopoulos P, Sutherland AD, Tang I, Taylor PL, Thaker M, Wang W, Yan M, Yu T, Wright GD.** 2013. The comprehensive antibiotic resistance database. Antimicrob Agents Chemother **57:**3348 –3357. http: //dx.doi.org/10.1128/AAC.00419-13.
13. **Hendriksen RS, Bangtrakulnonth A, Pulsrikarn C, Pornreongwong S, Hasman H, Song SW, Aarestrup FM.** 2008. Antimicrobial resistance and molecular epidemiology of *Salmonella* Rissen from animals, food prod- ucts, and patients in Thailand and Denmark. Foodborne Pathog Dis **5:**605– 619. http://dx.doi.org/10.1089/fpd.2007.0075.

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14. **Carattoli A, Zankari E, García-Fernández A, Larsen MV, Lund O, Villa L, Aarestrup FM, Hasman H.** 2014. *In silico* detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother **58:**3895–3903. http://dx.doi.org/10.1128 /AAC.02412-14.
15. **Saputra D, Rasmussen S, Larsen MV, Haddad N, Sperotto MM, Aar- estrup FM, Lund O, Sicheritz-Pontén T.** 2015. 1. Reads2Type: a web application for rapid microbial taxonomy identification. BMC Bioinfor- matics **16:**398.
16. **Jackson BR, Tarr C, Strain E, Jackson KA, Conrad A, Carleton H, KatzL, Stroika S, Gould LH, Beal J, Mody R, Silk BJ, Chen Y, Timme R, Doyle M, Fields A, Wise M, Kucerova Z, Sabol A, Roache K, Trees E, Kubota K, Pouseele H, Holt KG, Klimke W, Besser J, Brown EW, AllardMW, Gerner-Smidt P.** 18 April 2016. Implementation of nationwide real-time whole-genome sequencing to enhance listeriosis outbreak de- tection and investigation. Clin Infect Dis http://dx.doi.org/10.1093/cid /ciw242.
17. **den Bakker HC, Allard MW, Bopp D, Brown EW, Fontana J, Iqbal Z, Kinney A, Limberger A, Musser KA, Shudt M, Strain E, Wiedmann M, Wolfgang WJ.** 2014. Rapid whole-genome sequencing for surveillance of *Salmonella enterica* serovar Enteritidis. Emerg Infect Dis **20:**1306 –1314. http://dx.doi.org/10.3201/eid2008.131399.
18. **Taylor AJ, Lappi V, Wolfgang WJ, Lapierre P, Palumbo MJ, Medus C,Boxrud D.** 2015. Characterization of foodborne outbreaks of *Salmonella enterica* serovar Enteritidis with whole-genome sequencing single nucle- otide polymorphism-based analysis for surveillance and outbreak detec- tion. J Clin Microbiol **53:**3334 – 3340. http://dx.doi.org/10.1128/JCM .01280-15.
19. **Schatz MC, Phillippy A.** 2012. The rise of a digital immune system. Gigascience **1:**4. http://dx.doi.org/10.1186/2047-217X-1-4.
20. **Pecora ND, Li N, Allard M, Li C, Albano E, Delaney M, Dubois A, Onderdonk AB, Bry L.** 2015. Genomically informed surveillance for carbapenem-resistant *Enterobacteriaceae* in a health care system. mBio **6:**e01030 –15. http://dx.doi.org/10.1128/mBio.01030-15.
21. **DeLeo FR, Chen L, Porcella SF, Martens CA, Kobayashi SD, Porter AR, Chavda KD, Jacobs MR, Mathema B, Olsen RJ, Bonomo RA, Musser JM, Kreiswirth BN.** 2014. Molecular dissection of the evolution of car- bapenem-resistant multilocus sequence type 258 *Klebsiella pneumoniae*. Proc Natl Acad SciUSA **111:**4988 – 4993. http://dx.doi.org/10.1073/pnas .1321364111.
22. **Conlan S, Thomas PJ, Deming C, Park M, Lau AF, Dekker JP, Snitkin ES, Clark TA, Luong K, Song Y, Tsai YC, Boitano M, Dayal J, Brooks SY, Schmidt B, Young AC, Thomas JW, Bouffard GG, Blakesley RW, NISC Comparative Sequencing Program, Mullikin JC, Korlach J, Hen-derson DK, Frank KM, Palmore TN, Segre JA.** 2014. Single-molecule sequencing to track plasmid diversity of hospital-associated carbapen- emase-producing