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General Interest

An Overview of Foodborne Sample-Initiated Retrospective Outbreak Investigations and Interagency Collaboration in the United States

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ABSTRACT

Foodborne outbreak investigations have traditionally included the detection of a cluster of illnesses first, followed by an epidemiologic investigation to identify a food of interest. The increasing use of whole genome sequencing (WGS) subtyping technology for clinical, environmental, and food isolates of foodborne pathogens, and the ability to share and compare the data on public platforms, present new opportunities to identify earlier links between illnesses and their potential sources. We describe a process called sample-initiated retrospective outbreak investigations (SIROIs) used by federal public health and regulatory partners in the United States. SIROIs begin with an evaluation of the genomic similarity between bacterial isolates recovered from food or environmental samples and clusters of clinical isolates while subsequent and parallel epidemiologic and traceback investigations are initiated to corroborate their connection. SIROIs allow for earlier hypothesis generation, followed by targeted collection of information about food exposures and the foods and manufacturer of interest, to confirm a link between the illnesses and their source. This often leads to earlier action that could reduce the breadth and burden of foodborne illness outbreaks. We describe two case studies of recent SIROIs and present the benefits and challenges. Benefits include insight into foodborne illness attribution, international collaboration, and opportunities for enhanced food safety efforts in the food industry. Challenges include resource intensiveness, variability of epidemiologic and traceback data, and an increasingly complex food supply chain. SIROIs are valuable in identifying connections among small numbers of illnesses that may span significant time periods; detecting early signals for larger outbreaks or food safety issues associated with manufacturers; improving our understanding of the scope of contamination of foods; and identifying novel pathogen/commodity pairs.

According to the United States Centers for Disease Control and Prevention (CDC), between 2009 and 2015, 5,760 foodborne outbreaks were reported in the United States which resulted in 100,939 illnesses, 5,699 hospitalizations, and 145 deaths (Dewey-Mattia et al., 2018). These outbreaks comprise only a small subset of the overall estimated 48 million foodborne illnesses that occur every year (9.4 million of which are linked to known pathogens) (Scallan, Griffin, et al., 2011; Scallan, Hoekstra, et al., 2011). When an outbreak involves a product regulated by the U.S. Food and Drug Administration (FDA), the CDC and FDA Coordinated Outbreak Response and Evaluation (CORE) Network coordinate federal resources and collaborate with state and local partners to engage in a joint response.

Traditionally, foodborne illness outbreak investigations begin with the identification of clusters of illnesses which are hypothesized to be related to a common source (vehicle) based on microbial subtyping. An epidemiologic investigation of illnesses in this cluster is then initiated to identify a suspect food vehicle and a traceback investigation, along with product and/or environmental sampling, is used to confirm the link between the illnesses and the suspect vehicle. Sample-initiated retrospective outbreak investigations (SIROIs), on the other hand, follow the reverse pathway (Chen et al., 2016). A SIROI begins with the identification of a possible vehicle, due to the isolation of a foodborne pathogen from food or environmental samples (Jackson, Stroika, et al., 2016; Jackson, Tarr, et al., 2016) with genomic similarity to isolates

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that comprise an illness cluster, usually determined with whole genome sequence (WGS) data. Epidemiologic and traceback investigations are then initiated to corroborate the laboratory link between these isolates.

The concept upon which SIROIs are based is not new. However, attempts to link nonclinical and clinical isolates previously were limited by issues related to the limited sensitivity and specificity of the subtyping method used at the time. Federal and state partners have made substantial progress in their efforts to improve foodborne illness cluster identification and outbreak investigations by standardizing and improving surveillance activities associated with laboratory reporting and methodology, epidemiologic data collection, and data sharing.

Chief among improvements to surveillance efforts has been the widespread adoption of WGS as the primary microbial subtyping method. A multiagency collaboration between state laboratories, CDC, FDA, and the U.S. Department of Agriculture Food Safety Inspection Service (FSIS) began in September 2013, with the commitment to performing real-time WGS on all U.S. *Listeria monocytogenes* (*L. monocytogenes*) isolates from patients, food, and the environment surrounding the food, typically a production facility or farm, and share sequencing data by uploading it to a public repository for analyses (Jackson, Tarr, et al., 2016). Since 2014, WGS has been the primary microbial subtyping method for foodborne bacterial pathogens at the FDA – all isolates from FDA samples of *L. monocytogenes*, pathogenic *Escherichia coli*, and *Salmonella* are sequenced. CDC and state health departments have also steadily been increasing the capacity for WGS of clinical isolates, such that in 2019, WGS officially became PulseNet's new primary microbial subtyping method for identifying and classifying bacterial foodborne pathogens. PulseNet is the national federal/state laboratory network for foodborne disease surveillance. WGS data, as will be described further in this manuscript, allow comparisons of higher resolution of the genomic content of isolates and are critical cornerstones of SIROIs. International partners are also increasing their WGS capacity, which allows for international collaboration and identification of possible vehicles using signals investigated across the globe.

In addition, readily available exposure data from standard case report forms, such as the *Listeria* Initiative (LI) questionnaire implemented by CDC in 2005 for all listeriosis illnesses (U.S. Centers for Disease Control and Prevention., 2018), have provided a repository of baseline food exposure data for *L. monocytogenes* cases. The information can be readily queried for general exposures, such as food products, included on case questionnaires and can give early clues to relationships between a suspected food vehicle and an ill person, as one of the initial steps in an SIROI, lessening the burden on state health departments to respond to requests for initial exposure information. Therefore, follow-up interviews can be more targeted if and when case patients are available. The combination of WGS and readily available epidemiologic data (especially for listeriosis) enhances the ability of federal and state partners to engage in SIROIs.

SIROI findings help to identify potential suspect sources where inspections can be initiated, or samples collected to determine if food products may be contaminated. It is important to emphasize that genetic linkages must be interpreted in the broader context of epidemiologic and traceback data before conclusions can be drawn about the source of any illnesses. Challenges such as identifying the same strain in multiple facilities or identifying a specific strain in a facility that does not distribute to states where cases reside demonstrate why these links do not always yield a confirmed vehicle and sometimes require investigation of multiple processing facilities. In addition, even once confirmation through epidemiological and traceback investigations have demonstrated that the likely vehicle, produced by the firm, was contaminated for the time period of interest, additional evidence is needed to determine if the firm is continuing to produce food under those previous conditions or if corrective actions have alleviated concerns. This is particularly true if there is a large period of time between when environmental or food samples were analyzed, and the occurrence of the clinical illnesses. Due to the rapid identification of suspect sources, regulatory partners can initiate an inspection at the time a genetic link is discovered between food/environmental samples and recent clinical isolates. Regulatory partners then evaluate the conditions of the facility as the epidemiologic and traceback investigations are ongoing to ensure that products are not produced under insanitary conditions and determine if any regulatory actions may be necessary.

Here, we present the various processes used to identify and investigate SIROIs, interpret the results, and take the appropriate public health and regulatory actions. We also highlight two recent SIROIs to demonstrate the process, as well as the challenges and limitations that characterize them.

The sample-initiated retrospective outbreak investigation process

Background. The term SIROI is defined as an outbreak investigation that is initiated by the recovery of isolates of pathogenic microorganisms from product or environmental samples (collectively referred to as "nonclinical" isolates), followed by the identification of a cluster of genetically related clinical isolates with subsequent efforts to collect epidemiologic and traceback data to confirm a link between a suspect vehicle and illnesses. The terms used in this paper to describe SIROIs are defined in Table 1. Additional descriptions of various aspects of this process, including by Brown et al. (2019), are available (Brown et al., 2019). SIROIs begin with the recovery of an isolate of a pathogen from a food product or environmental sample. Using WGS, this isolate is compared to public databases, such as NCBI, containing clinical isolates of the same pathogen, allowing for the identification of potential retrospective clusters. Retrospective clusters are then triaged to determine whether further epidemiologic investigation should be initiated. Evaluating the available evidence for these clusters includes the following: 1) determining the source of the food or environmental isolates, the description of the product, and the regulatory history of the associated firm and potential for regulatory activities, 2) assessing its connection to other isolates through the formation of subclusters, and 3) assessing the likelihood of success for an epidemiologic investigation (based on a variety of factors such as availability and quality of exposure information, the number and timeframe of illnesses, etc.).

If epidemiologic and/or traceback investigations are initiated, their findings are analyzed to determine whether they support the link between the nonclinical isolate and clinical isolates. Specifically, this involves examining food exposure information and traceback or trace forward information to confirm whether the data support the hypothesis that the product/facility source of the isolate caused the illnesses in question.

Sample collection and sources. The process of collecting and analyzing product and environmental samples, recovering pathogenic isolates, sequencing their genomes, uploading the data to a publicly available national repository, performing comparative analyses of isolates, and taking action based on that information has been described previously (Pightling et al., 2018). Several scenarios can yield food and environmental isolates, which feed into this process. State and federal food regulatory agencies routinely and regularly conduct inspections and investigations at food manufacturing facilities, which may involve the collection of environmental and food samples. During for-cause inspections, commodity-specific assignments, or risk-based prioritization, environmental samples are collected from manufacturing plants or commercial kitchens. Finished food product samples may also be collected throughout the distribution chain and at retail establishments as part of investigations. Regulatory agencies also conduct surveillance sampling, in which a large number of samples of targeted foods are collected to ascertain the prevalence of microbiological contamination; surveillance assignments can target both imported and domestically produced foods (U.S. Food and Administration, 2019).

Table 1
Definitions of terms used in sample-initiated retrospective outbreak investigations

Term	Definition
Sample-Initiated Retrospective Outbreak Investigation (SIROI)	Sample-Initiated Retrospective Outbreak Investigations are initiated by the recovery of isolates of pathogenic microorganisms from product or environmental samples, followed by the identification of a cluster of genetically related clinical isolates with subsequent epidemiologic and traceback investigations confirming a link between a suspect vehicle and illnesses.
Traditional outbreak investigation	Traditional outbreak investigations are initiated by the detection of a cluster of illnesses, followed by an epidemiologic investigation identifying a suspect food item, and traceback and/or sampling supporting a link between the suspect vehicle and illnesses.
Foodborne illness outbreak	An incident in which two or more people experience a similar illness resulting from the ingestion of a common food.
Environmental samples	Samples from the environment surrounding the food, typically in a production facility or farm.
Food samples	Samples of food products ready to go to market, as well as in-process and raw ingredient samples.
Clinical isolates	Bacterial isolates recovered from human specimens.
Genetic relatedness	A measure of comparison for describing the extent to which bacterial isolates are genetically related to each other. (Pightling et al., 2018)
Traceback	Process of reviewing product supply chain records to identify the origin of food served or sold at a specific point of service.
Records	Documents that confirm the movement of food from one place to another along the supply chain. Records consist of a wide variety of types, including invoices, purchase orders, bills of lading, production logs, etc. Records can be stored in paper or electronic format.
Supply chain	The path a food takes from origin to point of service. Specific points along the supply chain include but are not limited to grocery stores, restaurants, distribution centers, processors, manufacturers, farms, and fields.
Timeframe of interest	The time period when contaminated product could have moved through the supply chain. This is often calculated by considering both the shelf life of the product and the product turnover rate at various points along the supply chain.
Resident strain	A bacterial strain especially *L. monocytogenes* that has become established in a specific location in a food facility environment over time.
Corrective actions	Corrective actions are the actions that must be taken if a critical limit is exceeded at any step of food production in a food business (e.g., delivery, storage, preparation).
PulseNet	PulseNet is a national laboratory network that connects foodborne illness cases to detect outbreaks, using DNA fingerprinting of bacteria making people sick.

Research projects are another source of sample collection. Sample isolate sequences uploaded for these purposes are commonly collected by federal, academic, and industry partners. Industry may also voluntarily contribute sequencing data from isolates identified via their own monitoring of the manufacturing environment, ingredient, and finished product sampling.

Bacterial isolate subtyping methodology. As previously mentioned, prior to the implementation of WGS as the standard molecular subtyping method, pulsed-field gel electrophoresis (PFGE) was used by public health laboratories and widely recognized as the gold standard microbial subtyping method for foodborne disease cluster detection. PFGE was crucial in detecting and responding to widely disseminated, multistate foodborne illness outbreaks, and investigation of these outbreaks ultimately resulted in food safety improvements and declining illness incidence (Kubota et al., 2019). The more common a PFGE pattern, the more illnesses were required to detect a potential common source outbreak represented as an increase of cases beyond the baseline or expected rate for that pattern. In addition, the lower resolution of PFGE sometimes resulted in misclassification of isolates into potential clusters when in fact no true common source existed, because the PFGE banding pattern appeared indistinguishable. The opposite misclassification scenario, in which isolates that were highly genetically related were linked to a common source, but yielded slightly different PFGE pattern combinations, also occurred (Brown et al., 2019).

WGS minimizes misclassification errors associated with PFGE and provides a more accurate grouping of isolates into likely common source clusters (Brown et al., 2019). While PFGE looks at differences at a limited number of restriction enzyme sites, WGS looks at specific differences between thousands of loci, providing more discriminatory power for inferring genetic relatedness between isolates (Stevens et al., 2022). In addition to its impact on the ability to identify traditional clusters, the expanded use of WGS to classify and catalog recent and historical isolates allowed bioinformaticians to compare isolates in novel ways, one of which is the use of SIROIs. A SIROI is defined based on the order in which the investigational information is obtained, and the initial trigger for the investigation (Fig. 1). SIROIs begin with the isolation of a pathogen from a food or environmental product, which is then uploaded to a public sequencing database. Bioinformaticians then compare the isolate data within the database in search of clinical isolates that could be considered genetically related to the food or environmental isolate. As opposed to traditional outbreak investigations, SIROIs begin with the possible vehicle; if a cluster of clinical illnesses is identified as being potentially genetically related to that vehicle, investigational partners may then employ subsequent epidemiologic and traceback investigations to confirm the vehicle as the cause of those illnesses. This approach allows for the determination of potential associations between foods and very small numbers of human illnesses or human illnesses with disparate temporal distribution, often prior to the point at which traditional outbreak detection methodologies would identify them as potential common source clusters.

SIROIs may entail several different scenarios with regard to temporal relationships between isolates, with human illnesses occurring during the same time period, before, or after the related food/environmental isolates were detected. Regulatory and public health agencies have collaborated to develop a SIROI process that considers a variety of factors to determine whether to pursue further epidemiologic and traceback investigations of these retrospective clusters, as presented below.

WholeGenome Sequence analysis. Bacterial isolates are further characterized by WGS. Genomic data are added to the National Center for Biotechnology Information (NCBI) Pathogen Detection database that is maintained as part of the U.S. National Library of Medicine by the National Institutes of Health. The database acts as a repository for microbial isolate information that has been submitted by many domestic and global public health agencies, regulatory agencies, universities, and research institutions. NCBI's Pathogen Detection database is the primary repository for data generated by the GenomeTrakr Network, which is an international collaboration of government and academic laboratories (Pightling et al., 2018).

After the WGS information on an isolate is uploaded to NCBI, it can be compared to other isolates already in the database. A preliminary examination of the data is performed as part of the Pathogen Detection workflow, which includes whole-genome multilocus sequence typing (wgMLST), single-nucleotide polymorphism (SNP), and phylogenomic analyses. The results are closely monitored for the presence of similar

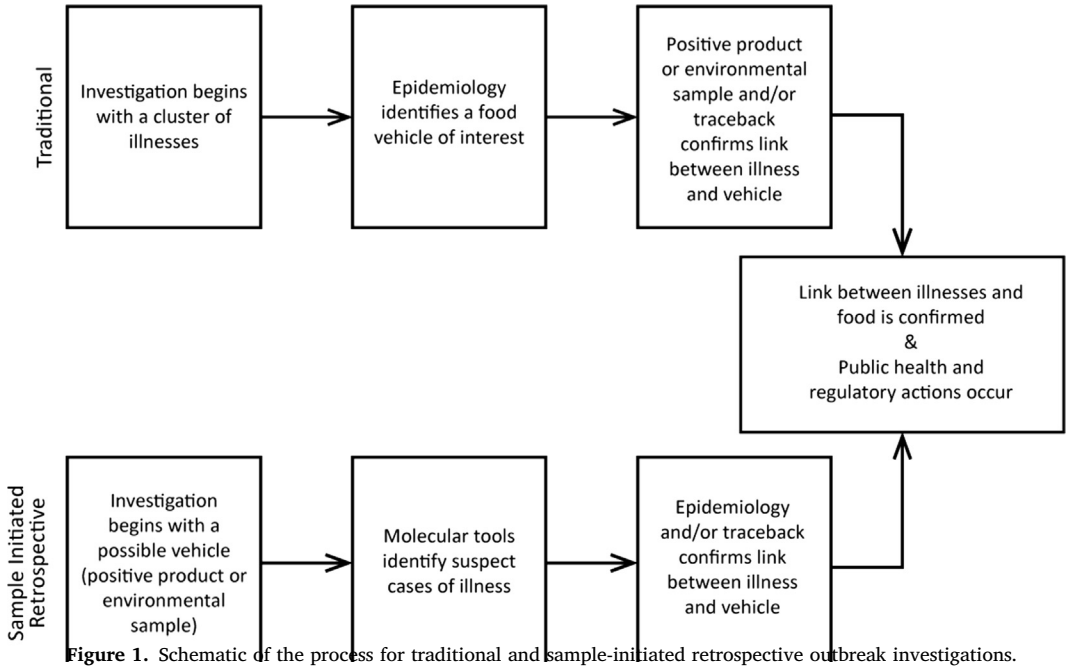

Figure 1. Schematic of the process for traditional and sample-initiated retrospective outbreak investigations.

sequences from other food samples, environmental samples, or clinical samples (Table 1). If the uploaded isolates' WGS information is genetically related to those that are present in the database, further analysis is performed. This includes examining the sources of genetically related isolates, as well as other metadata, and comparing them to the isolate of interest. This process has been described in detail elsewhere (Pightling et al., 2018). It is possible, therefore, to identify potential linkages between the product or environmental isolate to clinical isolates. These clinical isolates may have been identified prior to, contemporaneous to, or after the product or firm isolates.

FDA Retrospective Cluster Evaluation. Once a food or environmental isolate is determined to be genetically related to clinical isolate(s), FDA begins evaluating information for the product or firm's production environment to determine if a possible relationship may exist between the isolates in the cluster. FDA evaluates and inquires about additional sample information which may include where the sample was collected; date that the sample was collected; the firm where the food was manufactured, processed, packed, or held; any historical information related to the firm's production practices and previous sample findings; and any additional information about products that may be subject to contamination at the firm. FDA epidemiologists also review available clinical information for those isolates genetically related to the food or environmental sample(s). Evaluation includes reviewing the isolation dates for ill people (the date that the bacterial isolate was recovered from a clinical specimen), food or environmental sample collection dates, and geographic distribution.

FDA's investigation may further characterize the relationship between the food or environmental samples and the clinical isolates, establishing a hypothesis for a possible firm of concern or contamination source. After FDA's evaluation of the relationship between isolates, a hypothesis may be generated about a potential vehicle that caused the persons to become ill. While the evaluation may not identify a specific firm, it may provide relevant clues to further investigate a particular food commodity, geographic location, or group of firms. Once these targets are determined, FDA may consider additional sample collection (product and/or environmental) or initiating an inspection at the target(s). This will allow FDA to better determine if these targets may be the source of contamination, leading to persons becoming ill, while also determining if food currently being manufactured, processed, packed, or held may be contaminated and of ongoing public health concern.

Interagency Collaboration. Once information on the nonclinical and clinical isolates is evaluated and a possible hypothesis about their linkage is developed, FDA shares these findings with CDC so that the two agencies may collectively assess and develop vehicle hypotheses, as well as determine appropriate next steps. CDC's PulseNet team reviews the genetic relatedness by evaluating the isolates' relationship to other isolates within the PulseNet national databases. A discussion between both agencies' bioinformaticians, database analysts, and epidemiologists occurs and depending on the outcome of those discussions, an epidemiologic investigation may be further pursued. For some pathogens such as *L. monocytogenes*, CDC may already have exposure information on-hand from ill-person interviews conducted by state partners. Often, even with preliminary information available, more specific food exposure information may need to be requested from state or local health departments, including more details on the type, brand, or other related description of the item in the patient's food history.

When a retrospective cluster is identified and deemed appropriate for further epidemiologic and traceback investigation, public health partners may pursue patient food exposure history through several methods. Traditionally, exposure information is collected through interviews with ill people, and other technologies to collect electronic consumer data. Grocery shopper/loyalty card records, credit/debit card information, purchase receipts, and smart phone applications are being used to gather information about food purchases. With the additional clues associated with the matching food or environmental isolate in a retrospective cluster, investigators can reach out to ill people and retailers to request purchase documentation during the exposure timeframe. Records are evaluated for purchases potentially associated with the related food product, manufacturer, or brand. If a purchase transaction of interest is identified, this may be an opportunity for inclusion in a traceback investigation, and further record review to determine if illnesses can be linked to a common source, and to the food supplier or manufacturer associated with the nonclinical isolate.

Regulatory Considerations. Throughout the SIROI process, the regulatory strategy is being constantly evaluated. While SIROIs may

determine that a processing breakdown happened, current inspectional or sample evidence is critical in determining any ongoing product or processing concerns. If FDA and CDC have a working hypothesis targeting a specific food or firm, transparent communication will occur about any findings of interest. If FDA is aware that food currently being produced and available for consumers may be contaminated, the Agency has regulatory options to ensure that consumers are not at risk. The regulatory options may include a warning letter, injunction, seizure, import alert, and recall of the implicated product.

At the time of the product or environmental positive sample, FDA will communicate our findings to implicated food producers. A prudent processor should have taken corrective actions to eliminate any potential sources and routes of contamination when the initial positive samples were collected. If FDA deploys additional resources to conduct inspections or collect samples, and determines additional food may be contaminated, FDA will communicate these additional findings and may request the firm evaluate their process and voluntarily remove implicated products from the market. The determination and scope of products of concern may be impacted by WGS results suggesting a resident strain in the firm, the ill persons confirmed (through epidemiologic and traceback investigations) to have eaten the firm's food, shelf life of the implicated products, and processing conditions and equipment usage within a processing environment. While FDA is evaluating the retrospective outbreak and the conditions in the firm, a dialogue is occurring with the firm about any corrective actions taken since the previous positive sample or other actions which may alleviate FDA's concerns about product contamination. Data detecting and correcting any potential sources and routes of contamination in the processing environment are necessary to evaluate the conditions in the food processing environment. Since a SIROI signifies a systematic breakdown, FDA then expects a product processor to effectively manage the risk of future contamination by seeking and destroying any sources or routes of contamination, reacting to any environmental positive results, and remaining vigilant to any risks with the process or product. Failure to ensure food is produced in a safe environment will lead FDA and other regulatory partners to consider additional regulatory options to protect public health.

Two Case Studies of Recent Retrospective Foodborne Illness Clusters and Outbreak Investigations. To demonstrate this process, we present two case studies of recent SIROIs linked to the production and consumption of ice cream and cake mix. These outbreaks required the mobilization of a significant number of public health professionals and resources at the local, state, and federal level to protect public health and prevent additional illnesses. Additionally, evidence produced during the SIROIs led to product recalls, facility inspections and sampling activities, and communications to warn the public about the recalled products.

Investigation of *Listeria monocytogenes* Illnesses Linked to Ice Cream. In August 2017, as part of an FDA sampling assignment designed to verify industry compliance and gather baseline environmental surveillance data and inspectional information on ice cream facilities, FDA conducted an inspection at Ice Cream Producer A (Allard et al., 2019; U.S. Food and Administration, 2022). The inspection resulted in observations of insanitary conditions and *L. monocytogenes* was isolated within the facility's processing environment. These *L. monocytogenes* isolates were sequenced, and their genomes were uploaded to NCBI. WGS demonstrated that FDA's environmental sampling isolates were genetically related to two 2013 clinical isolates from the same state, representing infections that occurred in 2013. In response to FDA's findings, the firm conducted a voluntary recall in October 2017 and the Agency conducted a regulatory meeting in which the firm committed to implement corrective actions.

In August 2018, FDA determined a recent 2018 clinical isolate was genetically related to Ice Cream Producer A's 2017 environmental isolates and the two 2013 clinical isolates. FDA notified CDC of this link and requested information on any ice cream exposures of the three ill people. Based on the genetic relatedness of the clinical isolates to the environmental samples from Ice Cream Producer A, FDA also initiated a follow-up inspection at the manufacturing facility in September 2018 and determined that the firm had not implemented corrective actions following the 2017 regulatory meeting. The Florida Department of Health (FL DOH), in collaboration with CDC, collected food exposure histories from ill people related to ice cream consumption, and conducted a traceback investigation for the three listeriosis illnesses. The three illnesses of listeriosis were comprised of elderly individuals residing in long-term care facilities (LTCFs), who regularly consumed ice cream. Of the two individuals who became ill in 2013, the LTCF associated with one case specifically indicated that the patient consumed ice cream manufactured by Ice Cream Producer A. The individual who became ill in 2018 reported regularly eating scoops of vanilla and chocolate ice cream from Ice Cream Producer A. FDA was able to provide trace forward information (product and distribution from the facility) to compare with the results of the traceback investigation. The combination of the epidemiologic, laboratory, and traceback evidence collected demonstrated a strong link between the illnesses and the ice cream products manufactured by Ice Cream Producer A. The traceback investigation confirmed that during the timeframe of interest, the ice cream available at the residential facility was manufactured by Ice Cream Producer A. Ice Cream Producer A sold around 40% of its products to nursing homes and assisted living facilities, highlighting the concern for the risk their product posed to the elderly and immune-compromised, given the likelihood of contamination by *L. monocytogenes*.

FDA's 2018 follow-up inspection at Ice Cream Producer A again resulted in the recovery of *L. monocytogenes* isolates from their processing environment, specifically on food contact surfaces, and insanitary conditions were again identified that could have led to contamination of food products. WGS analysis confirmed that the newest *L. monocytogenes* isolates collected from the environment of Ice Cream Producer A facility in 2018 were closely genetically related to the isolates obtained from the three ill people and the older environmental isolates from 2017. Based on the cumulative findings, FDA and CDC concluded that Ice Cream Producer A was the source of the food that caused illness in these three individuals. FDA, CDC, FL DOH, and the Florida Department of Agriculture and Consumer Services communicated their concerns to the firm, which resulted in the firm voluntarily recalling all ice cream products manufactured between August 2017 and October 2018. On October 19, 2018, FDA suspended the food facility registration of Ice Cream Producer A.

This example illustrates a successful use of the SIROI process from the point when it was initiated in 2018, after a recently uploaded clinical isolate was found to match the strain of the historical environmental and clinical isolates. This investigation involved ill people that were good food historians or had readily available surrogates that recalled their exposure to the outbreak vehicle; one of the interviews was conducted with nursing home staff. This example highlights the importance of standardized questionnaires, the use of surrogates for food information, and the collection of data such as whether a case resided at an LTCF or had a previous hospitalization, and how helpful that information is to identify a food vehicle, such as ice cream, which is served frequently in hospitals and LTCF.

At the time when the 2017 environmental isolates were identified and determined to match the 2013 clinical isolates, the SIROI process was under development. While an outbreak investigation was not initiated at the time, FDA pursued regulatory activities in 2018 based on the inspectional findings. In general, SIROIs are often prioritized for clusters that include recent illnesses.

Investigation of *Salmonella* Agbeni Illnesses Potentially Linked to Cake Mix. In August 2018, the Oregon Health Authority (OHA) collected a box of Brand A white cake mix at retail as part of a traditional multistate outbreak investigation in collaboration with CDC (Ladd-Wilson et al., 2019; U.S. Food and Administration, 2019). A private

laboratory isolated *Salmonella* Agbeni from this cake mix, which did not match the outbreak strain that OHA was investigating at the time. However, WGS analysis demonstrated that it was genetically related to several other recent clinical isolates of a different strain. To explore this potential link between the cake mix isolate and clinical illnesses, CDC PulseNet identified a potential common source cluster in October 2018. This SIROI was spurred by the food isolate obtained from a sample collected as part of a separate, completely unrelated outbreak investigation. Ultimately, the cake mix sample's genetic characterization aided the hypothesis development for this investigation. The *Salmonella* Agbeni cluster initially included 15 cases and was identified based on PFGE matching the cake mix, since not all of these case-isolates had been sequenced at this point. As sequencing information became available, the isolates of interest were refined to focus on a subcluster of isolates from seven ill people from Florida, Maryland, Missouri, Ohio, and Wisconsin, which were genetically related to the Brand A white cake mix isolate by WGS.

Unfortunately, limited epidemiologic information was available. Further investigation indicated that two ill people reported eating cake prior to illness onset and another person reported eating raw cake mix, but brand information was not available. Food exposure information, including cake mix, was not available for the three ill people, and one patient denied any cake mix exposure. Manufacturer A was informed of the positive cake mix sample collected by OHA and its potential link to clinical illnesses, and as a result, issued a recall of various cake mixes. In late October 2018, FDA inspected the manufacturer of the recalled cake mix and collected additional finished product, ingredient, and environmental samples; all tested negative for *Salmonella* spp. The firm made voluntary corrective actions to address the positive sample, and no further regulatory action was taken. Ultimately, the epidemiologic and traceback information available was not sufficient to conclusively identify this cake mix as the vehicle for the outbreak, or to determine that this outbreak was caused by contaminated cake mix(es) produced by Manufacturer A. While the investigation was able to identify cake mix as the suspect vehicle leading to a product recall and public messaging to protect public health, the epidemiologic investigation could not confirm the link due to the lack of brand information. The recall may have prevented additional illnesses, since cake mix is a shelf-stable food, and consumers could have consumed potentially contaminated product well after the investigation. At the time of the investigation in 2018, PFGE was still the primary subtyping method for clinical isolates of *Salmonella*, which resulted in the initial inclusion of cases in the investigation that were later determined to be unrelated when more specific WGS data became available.

Challenges and limitations of retrospective outbreak investigations

Limited Resources and Evidence. Although the examples described previously met with different levels of success, they were similar in that an epidemiologic investigation was initiated after review of the initial laboratory findings. However, the majority of potential retrospective clusters fail to meet this threshold. One of the most common scenarios occurs when isolates from environmental or product samples are recovered as part of a sampling assignment at the retail level or an inspection at a food facility and are subsequently linked by sequencing to clinical isolates, but no epidemiologic investigation is pursued. Further investigation may not be pursued due to factors such as the length of time between the clinical and nonclinical isolates, how long ago the clinical isolates were obtained, how genetically related the isolates are, and the likelihood that an individual would be able to remember consuming a contaminated product or a product produced by a firm with a positive environmental sample. Specifically, in the case of listeriosis outbreak investigations, cases are often significantly spread out temporally, by weeks, months, or in some cases, even years. It is important for the suspected food vehicle to be specific enough so that it is available on the LI questionnaire, such as ice cream or particular cheese types. Otherwise, reinterviews become increasingly challenging. These factors all relate to the relative likelihood of success of an epidemiologic investigation given limited public health resources. Because of the way in which the public health system in the United States is structured, state and local health departments have a wide variety of responsibilities and must weigh the potential impact of further investigating illnesses identified in a retrospective cluster with competing priorities. Even when an epidemiologic investigation is initiated, it may fail to produce highly specific exposure information needed to conclusively identify a food vehicle.

Ready-to-eat (RTE) food products present a variety of complexities as RTE foods often have multiple ingredients, may involve post-processing contamination, potential source contamination at growing or harvesting (e.g., produce or RTE seafood) without a kill step, and contamination between suppliers through multiple ingredients. Epidemiologic information collected from ill people with clinical isolates matching the product/environmental isolate may not correspond to the positive food product in question if that product was used as an ingredient in the actual food vehicle that made the individuals sick. For example, previously, multiple *L. monocytogenes* isolates were isolated from environmental and food samples from an RTE deli salad and sandwich manufacturer. Although there were clinical isolates that were found to be genetically related to these food and environmental isolates by WGS, no epidemiologic or traceback link to the manufacturer was identified. Ill people with this same strain continued to become infected over several years, and eventually, an environmental isolate from a different manufacturer that produced RTE hard-boiled eggs provided additional clues to the investigation. The outbreak was ultimately linked to hard-boiled eggs produced by this other manufacturer (U.S. Food and Administration, 2020).

These challenges are often compounded by the fact that food and environmental isolates may be submitted to the public database with minimal metadata (additional data regarding sample or product type, product brand, or location), and often regulatory agencies have limited avenues to access information related to specific brands, firms, type, location, and collection conditions. As a result, public health authorities have limited information about the isolates to help inform an epidemiologic investigation, which can result in a lack of prioritization. This may prevent a source from being identified and confirmed for those cases, as described previously.

Quality of Epidemiologic and Traceback Data. To reach a successful outcome, a potential link between a food or environmental isolate and genetically related human isolates must be validated by supporting epidemiologic and traceback information. Even if circumstances align such that public health authorities agree to initiate an epidemiologic investigation to obtain this supporting information, there is no guarantee that they will be successful. The same challenges that plague epidemiologic investigations in routine foodborne outbreaks still apply. For example, interviews may fail to identify a novel exposure if the right questions are not asked. The length of elapsed time between interview and potential exposure, as described previously, can also impact the successful recollection of specific food items. Shopper card and credit card information, when available, may be able to track some exposures, but this information is not always available or detailed enough.

In addition to epidemiologic evidence, traceback evidence is also often necessary to confirm that illness represented by clinical isolates can be attributed to the product or firm yielding the positive sample in question. As with epidemiologic investigations, traceback challenges encountered during SIROIs are similar to those encountered during traditional outbreak investigations (Marshall et al., 2020). One of the challenges includes the limited availability of epidemiologic information required to initiate a traceback investigation, which can lead to significant delays in the overall investigation. This is particularly

difficult for traceback investigations involving outbreaks linked to the consumption of fresh produce, such as leafy greens and avocados (Irvin et al., 2021; Marshall et al., 2020; Pomeroy et al., 2021). Additionally, the potential for limited product-identifying information throughout the supply chain requires investigators to take a step-by-step approach to product tracing, and thus creating inefficiencies and delays obtaining tracing data. Record-keeping issues across the food industry include poor record maintenance, difficulty of record interpretation, or simply lack of records altogether, especially during a SIROI that may take place years after the food has made its way through the supply chain and to the consumer. In some situations, a food product may have been discontinued and no longer be sold by the firm or specific records may not be available for collection because they were discarded by the firm. Finally, comingling of food ingredients and products can complicate traceback investigations because conducting traceback for multiple production lot codes coupled with numerous farms or facilities supplying each lot may result in the identification of many end points, making it difficult to determine a contamination source.

Food Supply Chain Complexity. Aside from epidemiologic limitations, the interconnectedness and complexity of the food supply chain, combined with the limited scope of baseline testing and prevalence data for pathogenic bacteria in a wide variety of food products, may also obscure the true source of clinical illness. Contaminated ingredients and foods may seed multiple processing facilities as they move through distribution channels, such that the immediate cause of an individual illness is not necessarily the same as the product or firm currently in question. While WGS confirms that a relationship between isolates exists, it does not necessarily illuminate the specific causal nature of that relationship. An additional challenge includes the identification of genetically related nonclinical isolates recovered from environmental samples in a facility that manufactures multiple food products.

For all these reasons, many more potential linkages between clinical and nonclinical isolates (potential retrospective clusters) are identified than yield actual SIROIs. Furthermore, even those SIROIs that occur do not always result in confirmation of a food vehicle, or enough evidence to determine conclusively that the product (or firm) of interest caused the illnesses in question. However, public health authorities recognize that initiating SIROIs to gather supporting epidemiologic and traceback information are critical to interpret linkages between illnesses and foods or environments and can lead to substantial benefits to public health.

Opportunities and impact of sample-initiated retrospective outbreak investigations

Impact to Outbreak Response. Because of the precision of WGS, even single cases may be attributed to a specific food product (something that is rarely possible without a sequencing link) (Jackson, Stroika, et al., 2016). SIROIs allow for the identification of firms that are responsible for outbreaks of even a very small number of illnesses, which might not have been identified by traditional cluster investigation techniques. Investigation of sample-initiated retrospective clusters can help identify novel or unusual food vehicles (Crowe et al., 2017; Thomas et al., 2020). SIROIs can also lead to faster identification of the source of an outbreak by providing a confirmed laboratory signal about the potential vehicle from the beginning of the investigation (compared with traditional investigation methods that must deduce this signal from case patient interviews). Earlier identification of novel pathogen/commodity pairs allows food safety authorities to take appropriate regulatory and public health actions, as well as targeted education and outreach to address possible food safety knowledge gaps by industry partners and the consumer. SIROIs also allow for better targeting of resources by federal regulatory agencies by eliminating some of the uncertainty inherent in traditional outbreak investigations in which the vehicle is unknown. Finally, SIROIs allow for reinvestigation of older outbreaks that were unsolved by providing new commodity hypotheses when firms are discovered to have sample isolates that sequence into historical SNP clusters/trees. The microbial hazards of these commodities may be underestimated, and a firm may be more closely evaluated in follow-up inspections and regulatory activities.

Benefits to Industry. The nature of SIROIs lends itself to the analysis of persistent pathogen contamination in facilities that is of public health concern (Brown et al., 2019). At the most fundamental level, the detection of retrospective illness clusters demonstrates that the bacterial pathogens present in a firm or in products produced by that firm are capable of causing human illness. Successful SIROIs take this one step further and prove that the firm's products actually caused illness. A potential or confirmed link to human illness can underscore the urgency of contamination issues, prompting firms to evaluate if additional food could be contaminated or processing conditions may lead to product contamination. As already described, SIROIs allow for this determination to be made with fewer illnesses, so that firms can take steps to address microbial contamination issues before they cause large- scale outbreaks. Some firms already use WGS themselves to proactively identify contamination issues within their facilities so that they can then target these issues with corrective actions to prevent contamination of their products. In addition, firms who have had isolates collected from food samples or environmental surfaces, which are genetically related to isolates from other firms' food or environmental surfaces, may reevaluate their supplier safety protocols. A common supplier may be the cause of a similar and shared pathogen across firms and industries. Through this recognition, they can potentially reduce the number of contaminated products from entering the market, either by changing suppliers or working with their existing suppliers to address microbial contamination issues. Underlying all these benefits to industry posed by WGS and its application in SIROIs, is the open and transparent nature of these data, which empowers industry themselves to engage in retrospective analysis to make their own independent assessments (Lambert et al., 2019).

Foodborne Illness Attribution and Policy Implications. The SIROI process enhances investigational partners' ability to attribute illnesses and outbreaks to specific food commodities. More complete and thorough data on foodborne outbreaks enhance all aspects of research into the epidemiology of foodborne illness. Increasing the number of outbreaks with a confirmed pathogen source and pathway may also help elucidate the overall burden of foodborne disease by improved linkage of outbreaks to specific foods. As already described, these data may help explain new, previously unidentified, or rare pathogen/commodity pairs and contribute to the development of new public guidance or food safety policies. Development of interventions and allocation of finite resources by industry and food regulatory agencies relies on this information. Furthermore, SIROIs allow for the attribution of small numbers of illnesses that previously would have been characterized as sporadic or "baseline" illnesses with no cause identified. Reducing the extent of sporadic or baseline cases due to unknown sources further improves the collective understanding of which foods can cause human illness, and the corresponding development of appropriate food safety polices and guidance.

Food Safety Research Implications. The findings of SIROIs provide information that can enhance the collective understanding of pathogen/food dynamics. For example, analysis of data generated by SIROIs expands our understanding of the temporal distribution of foodborne illness attribution estimates. This information is important in estimating the overall burden of foodborne illness and how it may differ over time. The attribution data generated by SIROI investigations can also be incorporated into risk assessments, which are used to analyze the relative risk of various commodities. An example highlighting these research opportunities is a SIROI linked to ice cream in 2015, which provided one of the first opportunities to assess the exposure levels to L. monocytogenes from the implicated ice cream prod-

ucts among infected people and the overall population (Pouillot et al., 2016). While the majority of people who were exposed to the product did not become ill after ingesting low levels of L. monocytogenes, highly susceptible people did, adding additional evidence of the greater risk for listeriosis faced by people with weakened immune systems. Additional research included the comparison of enumeration methods, determining the prevalence and enumeration data, recovery, and growth potential from milkshakes from the naturally contaminated ice cream products, and enrichment dynamics of the pathogen using three different enrichment formulations (Chen et al., 2016; Chen et al., 2017; Chen et al., 2016; Ottesen et al., 2016).

International Collaboration. As more institutions and international agencies begin to contribute sequence data to international sequencing databases, such as NCBI, the abilities of public health and regulatory agencies around the world to detect illness clusters will be enhanced and this will aid in the development of earlier vehicle hypotheses and effective responses. For example, genomes from *Listeria* isolates recovered from enoki mushroom samples that were uploaded to NCBI by Canada in 2020 led CDC to reopen a multiyear listeriosis outbreak in the United States that had remained unsolved despite multiple investigations. The sampling ultimately linked enoki mushrooms imported from the Republic of Korea to illnesses spanning multiple years in Australia, Canada, and the United States (Pettengill et al., 2020). This scenario, in which a contaminated food is widely distributed internationally, resulting in illness spanning multiple countries, is not unique, as globalization of the food supply has increased over the last 15 years (U.S. Food and Administration, 2019). Just within the United States, approximately 10–15% of the U.S. food supply is imported, including 53% of fresh fruits, 29% of vegetables, and 93% of seafood consumed in the United States (U.S. Food and Administration, 2019). Because of the global distribution of food (and associated pathogens), outbreak investigations increasingly will require collaboration between international partners. Open sharing of genomic data is critical to ensuring the success of these collaborative investigations, whether they are SIROIs or traditionally detected outbreaks.

Conclusions

SIROIs are an important process that supports a prevention-centric focus on regulatory public health. SIROIs are not replacing traditionally detected outbreak investigations, but instead, provide an alternative pathway to protect public health. They allow for earlier detection and possible intervention to address microbial contamination issues, better targeting of regulatory agency resources, and improving our understanding of pathogen/commodity dynamics. As a result, they also provide an important information stream for foodborne illness attribution and resource projects. These linkages are publicly available which promotes transparency and an enhanced emphasis on food safety culture, across all food safety partners, including public health and regulatory agencies and industry. Through SIROIs and open international genomic data sharing, we can continue to improve our understanding of how microbial pathogens behave in the global food system and further develop interventions and strategies to control them and improve food safety globally.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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