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Whole Genome Sequencing and its role in Food Safety

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Food safety is a global concern as unsafe food containing harmful pathogens or chemical substances causes several diseases ranging from diarrhoea to cancers. In 2010, 600 million foodborne illnesses and 420000 deaths occurred due to 31 global food safety hazards and the global burden of foodborne diseases caused by these 31 hazards was 33 million healthy years of life lost due to illness and death (WHO, 2015).

Unsafe food also negatively impacts the food security of nations and affect the economic development by restricting the exports. Hence, food safety is becoming a public health priority and Governments are making efforts in developing policies and regulatory frameworks, establishing and implementing effective food safety systems to ensure that food producers and suppliers along the entire food chain operate responsibly and supply safe food to consumers.

The epidemiological investigation of a foodborne outbreak, that includes identification of pathogen, source attribution, removal of contaminated food items from supply chain and development of other intervention strategies, depends on the ability to subtype the etiological agent at a high enough resolution to differentiate related from nonrelated cases. The traditional phenotypic subtyping methods used include serotyping, phage typing and biotyping.

Since 1990s, the field of subtyping was revolutionized with the advent of molecular and DNA based subtyping methods, which allowed more sensitive discrimination than traditional methods. Commonly used molecular subtyping/genotyping methods include banding pattern-based Pulse Field Gel Electrophoresis (PFGE), and DNA sequencing-based Multi Locus Sequence Typing (MLST), and Multiple Locus Variable number tandem repeat Analysis (MLVA). The establishment of PulseNet in USA in 1996 and its expansion as “PulseNet International” was instrumental in advancing the adoption of molecular subtyping for bacterial foodborne disease surveillance. PulseNet selected PFGE as the primary genotyping methodology which substantially advanced foodborne disease surveillance and outbreak investigation.

WHOLE GENOME SEQUENCING (WGS)

Whole Genome Sequencing (WGS) is a laboratory procedure that determines the complete DNA sequence in the genome of an organism in one process. Recent advances in sequencing technologies and bioinformatics tools have made WGS a viable and advanced solution for epidemiologic investigation and surveillance of foodborne bacterial pathogens (Deng et al. 2016). Due to advantages over PFGE, WGS is now becoming the preferred method for organism identification and comparison among isolates

CURRENT APPLICATIONS OF WGS IN FOOD SAFETY MANAGEMENT

(a) Regulators and Public Health Agencies

Whole Genome Sequencing (WGS) has already been used in few countries to subtype common foodborne pathogens wherein the high-resolution WGS subtyping data has enhanced the outbreak detection and facilitated epidemiological investigations. The high specificity and sensitivity of WGS provides greater confidence in regulatory decisions made by authorities on food safety, public health etc.

 USA

US FDA is utilizing WGS since 2008 and is coordinating efforts by federal, state, and international public health agencies to sequence pathogens collected from foodborne outbreaks, contaminated food products, and environmental sources and make their genomic sequences publicly available in a database called GenomeTrakr. GenomeTrakr, established by the FDA in late 2012, is the first distributed network of labs to utilize WGS for pathogen identification and can be used to help pinpoint the contamination sources of current and future outbreaks. As on Sep, 2016, this network has sequenced more than 71,000 isolates, and closed more than 175 genomes Since 2012, FDA is using WGS of foodborne pathogens for regulatory purposes in various ways (http://www.fda.gov): (1) Differentiating sources of contamination, even within the same outbreak. (2) Determining which ingredient in a multi-ingredient food harbored the pathogen associated with an illness outbreak (3) Narrowing the search for the source of a contaminated ingredient, even when the source is far off place (4) As a clue to the possible source of illnesses - even before a food has been associated with illnesses by traditional epidemiological methods.

WGS AND POTENTIAL ROLE IN FSMA COMPLIANCE

Under US FDA’s new Food Safety Modernization Act (FSMA), food industry is expected to have environmental monitoring programs (EMPs), especially when ready-toeat food is exposed to environment prior to packaging, to verify the effectiveness of their processing and sanitation controls in their processing plants. With several recent cases, wherein FDA has linked environmental positives in facilities from previous years with illnesses occurring today, food industry is expected to embrace WGS technology to check if they have resident strain of a pathogen in their ready-to-eat product facilities. Recently, California based Clear Labs Inc has released WGS test based on their proprietary next generation sequencing (NGS) platform. The test will allow food companies in identifying pathogen strains in samples, determine how different pathogen strains are evolutionarily related, what regions they come from, and from which food groups they originate.

 Canada

In Canada, WGS is being routinely applied in parallel to other methods like PFGE during cluster investigation or outbreak response; to monitor trends in emerging pathogens, anti-microbial resistance and to identify novel virulence factors. Canadian Food Inspection Agency is in the process of incorporating information from WGS into health risk assessments and epidemiological surveillance and the expansion of the PulseNet Canada laboratory network refers to the transition to include WGS as a primary subtyping method.

 Europe

Public Health England (PHE) has been using WGS routinely as part of specialist microbiology and epidemiology services and has benefitted from the application of WGS through improvements in surveillance and outbreak investigation. In June 2014, a WGS-based investigation by PHE identified the root cause of a Salmonella outbreak through eggs and prevented further outbreaks. By 2014, over 28,000 bacteria and virus genomes were sequenced including over 3,500 salmonella genomes (www.gov. uk). PHE is also using whole genome sequencing (WGS) to detect antibiotic resistance genes. In Denmark, food and environment monitoring for Listeria monocytogenes together with health surveillance using WGS was implemented in 2013. The efforts succeeded in linking sporadic cases over a long period and helped in identifying and

eliminating the outbreak source.

(b) Industry

Industry players like Mars, Nestle, ConAgra & DuPont are implementing WGS in their own food safety programs. Industry can use WGS to monitor ingredient supplies, to determine the effectiveness of preventive and sanitary controls and determine the persistence of pathogens in the environment. WGS can provide a much faster response time for outbreaks and recalls in the food industry.

INTERNATIONAL EFFORTS ON WGS FOR FOOD SAFETY

When compared to developed countries, the application of WGS for food safety management in developing countries has been very limited. In the developed countries, well established reporting system used by public health agencies is resulting in the proper documentation of incidence of foodborne illness in populations.

This reporting system is absent or almost negligible in the developing countries and there is a need to establish this reporting/ surveillance system in the developing countries before WGS can be effectively used in food safety management. The Food and Agricultural Organization (FAO) is leading global efforts to build capacity to detect, control, and prevent foodborne diseases.

As part of these efforts, FAO convened “Technical Meeting on the impact of WGS on food safety management within a One Health Framework” in May 2016 that was attended by participants from 50 countries (FAO, 2016). “One Health Framework” advocates the idea of fully connecting the clinical, food, and farm environments as an integrated whole rather than as disconnected parts. Global outreach for WGS in food safety management will require concerted efforts and coordination among various organizations like WHO, FAO, International Standards Organization (ISO) etc. There is a great need for training in WGS technology, its validation, harmonization, effective data-sharing and integration across the global community.

CAN INDIA MAKE STRIDES IN FOOD SAFETY WITH WGS?

Indian food industry has been adopting various Food Safety Management Systems (mostly private standards like FSSC 22000, BRC, SQF etc) largely to meet the customer /export country requirements. US FDA is already using WGS in narrowing its search for the source of a contaminated ingredient.

As part of the 2012 Salmonella outbreak investigation, FDA found that the Salmonella Bareilly DNA for the samples tied to the 2012 outbreak was very similar to the Salmonella Bareilly DNA isolated from shrimp that came from a processing plant in southwest India several years earlier.

With FSMA in force, US FDA is expected to increase its use of WGS to link environmental positives in ready-to-eat product facilities from previous years with illnesses occurring today. In next few years, there is possibility of WGS becoming mandatory for exports, specifically to few countries having WGS supported food surveillance systems in place.

This could affect the economy of several countries including India that do not have this technology for food safety. There is an imperative need for assessing India’s readiness in deploying WGS for food safety. For implementing WGS, India already has good infrastructure and pre-requisites in terms of sequencing capacity, high capacity storage & data analysis, good internet connectivity and bioinformatics support.

There are several research institutes in India with WGS facilities and research programs. There are also few private service providers for WGS, supporting research in agriculture and health care areas.

The major challenge for India to adopt WGS for food safety would be the database construction and the validation since foodborne disease surveillance in India is lacking. Foodborne disease surveillance is essential for monitoring of foodborne diseases and their trends, evaluating strategies for the control and prevention of foodborne diseases, detection etc.

India lacks systematic studies in understanding the types of foods involved and the agent causing the disease. Most of the foodborne disease outbreaks in India go unreported or are not investigated and some of them are only noticed after major health issue or economic loss has occurred.

The Centers for Disease Control and Prevention (CDC) in USA is example for an agency that is involved in continuous surveillance for foodborne disease outbreaks and provides valuable insights into the etiological agents, their variations and foods that cause illness and the environments where contamination occurs. The Food Safety and Standards (FSS) Act, 2006 stipulates the duties and functions of Food Safety and Standards Authority of India (FSSAI) which include regulating and monitoring the manufacture, processing, distribution, sale and import of food so as to ensure safe and wholesome food.

As per the act, the Food Authority shall also search, collect, collate, analyze and summarize relevant scientific and technical data particularly relating to (i) food consumption and the exposure of individuals to risks related to the consumption of food; (ii) incidence and prevalence of biological risk; (iii) contaminants in food. The Food Authority shall also promote, co-ordinate and issue guidelines for the development of risk assessment methodologies and monitor and conduct and forward messages on the health and nutritional risks of food to the Government.

Epidemiological, environmental, and clinical components should integrate closely for carrying out foodborne disease investigation. Routine collection and analysis of clinical, food, and environmental samples is a pre-requisite for implementing WGS. Hopefully, FSSAI will coordinate with the concerned agencies and take initiatives in building basic epidemiology, surveillance, food monitoring and testing infrastructure for implementing effective food safety systems in the country.

CONCLUSIONS

Whole Genome Sequencing (WGS) is a powerful tool that can be used for a wide range of public health and food safety applications. WGS is now being used to monitor emerging food pathogens and understand how pathogens spread within and between geographic areas. It can help the food industry to monitor ingredient supplies, determine the effectiveness of preventive and sanitary controls and determine the persistence of pathogens in the environment. The high specificity and sensitivity of WGS provides greater confidence in regulatory decisions made by authorities on food safety, public health as well as decisions made by food industry. However, basic foodborne disease surveillance systems need to be established in developing countries including India, before WGS can be used for food safety.

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