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Risk of Developing Antimicrobial Resistant *Listeria monocytoge*nes in India: A Short Narrative Review

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Abstract

Background and Objective: Rampant application of antimicrobial drugs in food sectors triggered the development of resistance within the microorganisms in the surrounding environment. Due to the reduced susceptibility towards existing drugs, these microorganisms have an increased survival rate when treated. The emergence of this complication in the common food-borne pathogens is worrisome. Several antimicrobial-resistant variants of known infectious bacteria have been discovered. *Listeria monocytogenes* is one among those 'superbugs' bringing such public health challenges to be tackled. This article aims to review India's current situation and stance regarding the progressive issue of antimicrobial resistance and listeriosis.

Results and Conclusion: The issue of antimicrobial resistance has been recognized at all food industry and health care domain levels. Solutions are constantly being made to combat the obstacle, but the antibiotic resistance crisis does not seem to retard. Despite the awareness, regulations, and restraints implemented across the globe, researches hint towards rising antimicrobial usage and the ensued more threatening infections. India's step towards curbing antimicrobial resistance is at par with other global policies and intends to lower the resistance development rate among all pathogens. Till now, Indian authorities and the public have shown insouciance towards listeriosis. There are no special rules targeting *Listeria monocytogenes* in India, as opposed to stringent regulations in many western countries. The Indian government and all associated authorities must study and develop plans to establish standards and statutes to control listeriosis. Above all, set up a surveillance system to monitor the causes of food-related illnesses across the country.

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1. Introduction

Food-borne illnesses are a significant public health threat. The World Health Organization (WHO) reported that approximately 600 million people are infected with a food-borne disease yearly[1]. An individual's health is hampered, but from a more comprehensive outlook, it also affects the overall socio-economic development of the population [2]. It strains the healthcare system and even harms the country's economic activities and trade. Chemicals, heavy metals, parasites, fungi, viruses all cause food-borne illness, but bacterial food infection cases are the common causation[3]. More than 90 percent of food poisoning is caused by *Staphylococcus aureus, Salmonella, Clostridium perfringens, Campylobacter, Listeria monocytogenes, Vibrio parahaemolyticus, Bacillus cereus,* and entero-pathogenic *Escherichia coli* every year [4]. *Listeria monocytogenes* is a food-borne bacterium. Table 1 summarises the food products likely to be contaminated by *Listeria*. *Listeria (L.) monocytogenes* is an opportunistic pathogen, meaning those with a low immune system are most likely to suffer from infection [9].

Table 1: Summary of foods potentially contaminated by *Listeria*. (Adapted) [5-8]

Foods likely to contain *Listeria monocytogenes*	References
Raw meat (chicken, mutton, turkey)	[5-8]
Sausages, Salamis	[5-7]
Raw/unpasteurized milk and its products	[5,7,8]
Soft cheese (Brie, Roquefort, Camembert), Fresh cheese (Feta, Ricotta)	[5,7]
Raw Seafood (Salmon, Shrimps, mussels)	[5,7,8]
Raw, unclean Vegetables and Fruits (leafy vegetables like lettuce, raw mushrooms, cantaloupes)	[5,7]
Unhygienically prepared food like sandwiches, deli meat recipes at retail shops	[5-7]
Frozen Foods, RTE packages	[5-7]

Invasive listeriosis, the severe form of the disease, affects high-risk groups of the population, including pregnant, elderly, infants, patients of cancer treatment, Human immuno-deficiency virus-Acquired Immune Deficiency Syndrome (HIV AIDS), organ transplants, or those with impaired immunity [10]. Even though the number of cases of listeriosis is small, the linked high mortality rate (20% -30%) makes it a concern; most require hospitalization [11,12]

The use of antibiotics in food-animal production is growing exponentially, especially in the Indian agriculture and flesh-food sector[13]. The excessive use of antimicrobials as prophylaxis and growth promoters has led to the emergence of antimicrobial-resistant bacteria and their rapid development of resistance [14,15]. The antibiotic susceptibility testing of *Listeria* isolates in many recent research studies revealed that most isolates were multidrug-resistant to antibiotics[6].

After distressing the western countries, listeriosis seems to be targeting the Indian population next. This study reviews the development of antimicrobial resistance (AMR) in the Indian environment, emphasizing *L.monocytogenes.* With this article, we aim to nudge public health organizations, food safety authorities, animal veterinary institutions, and related professionals to implement proactive measures to control the use of antimicrobial agents and prevent the additional spread of multidrug-resistant bacteria.

2. L. monocytogenes: general characteristics and listeriosis infection

For each million worldwide, 0.1 to 10 people are affected by listeriosis yearly, making it a comparatively rare food-borne disease [9,16]. Regardless, it is a serious concern due to the high mortality rate associated with this infection [12,17,18]. Twenty-three thousand one hundred fifty infection cases and 5463 deaths are caused by listeriosis every year worldwide [19]. Out of the five known species of *Listeria*, only *L. monocytogenes* have been proven to cause food poisoning; 4b serotypes are the major pathogenic strain [20,21]. In 1924 at Cambridge, England, listeriosis was first recognized by Murray and colleagues in laboratory guinea pigs and rabbits [20,21]. After a series of outbreaks in Europe and North America during the 1980s, it was well established that the intake of *Listeria*-contaminated food was the principal transmission method [7,22,23,24,25].

Listeria monocytogenes is a Gram-positive, non-spore producing, facultative anaerobe [6,7,26]. The optimal growth was exhibited between 35-37 °C. The strains show variability in their growth temperature and can grow even at 1 °C, thereby proliferating in refrigerated conditions [6,26-28]. When engulfed by macrophages inside a host, they efficiently break through the phagosome by hemolysin and phospholipase C and be freed [29,30]. It moves around from one cell to another by the "Actin Rocket" mechanism.[31,32] It accretes actin molecules at one end of its cell body, and this thrusts itself into another host cell where this process repeats, hence, invading farther and evading the immune mechanisms of the human body [31-33].

This pathogen is classified as low-grade, meaning if a low count of viable cells is ingested, then no clinical manifestations are observed in most healthy adults [34,35]. Broadly, the incubation period is nearly 14 days but can range from 4 to 70 days. This makes it difficult to establish links between positive cases and specific food exposures [24]. Infants, elderly, immune-compromised individuals (resulting from drugs or disease), and pregnant women are considered high-risk populations [7,9]. Abortion, septicemia, meningitis, and central nervous system infections are commonly observed threatening manifestations [7,28]. The symptoms of invasive listeriosis noted in the common population include diarrhea, fever, body aches, stiffness, mental confusion, and convulsions [7,9,25]. Researchers often emphasize *Listeria*'s ubiquity [6,36]. *L. monocytogenes* can be found in water, soil, vegetation and is frequently isolated from dairy and poultry products. Experiments on L. monocytogenes heat resistance showed that raising the internal temperature to 70°C for two minutes adequately destroyed the microorganism, reducing the risk of infection [37,38]. Due to various factors such as dietary habits, food processing methods, and health surveillance systems, listeriosis is prevalent in the American and European regions [39]. The first recorded outbreak in Canada was caused by

contaminated coleslaw in 1981 [40,41]. One of the most significant listeriosis outbreaks was in France (1992), with 279 cases and 63 deaths due to contaminated pork tongues [33]. Table 2 summarises some of the notable officially recorded listeriosis outbreaks worldwide. In 2007, 1557 confirmed cases were stated by EU countries [44]. France, Finland, Denmark, England, Belgium, and Wales saw an increase in the incidences, whereas a reducing trend was observed in Sweden. The US Centers for Disease Control and Prevention (CDC) stated that listeriosis alone accounted for approximately 1,600 illnesses and 260 deaths annually in the US [45].

Table 2. Documented listeriosis outbreaks and cases through the years across the globe

COUNTRY	YEAR	RECORDED CASES	REFERENCES
Australia	2012	26	[39]
Canada	1981	41	[7,28,42]
	2002	17	[7]
	2008	57	[39]
China	2009	14	[39]
	2011	13	[39]
	2012	26	[39]
	2001-2010	101	[43]
Denmark	1989-1990	26	[7]
European Union	2013	1763	[39]
	2015	2206	[6]
Finland	1999	25	[7]
	2012	10	[39]
France	1992	279	[7]
	1993	38	[7]
	2000	261	[43]
	2006	290	[43]
Germany	2005	510	[43]
	2006	508	[43]
India	1966	16	[42]
	1975	9	[42]
	1978	4	[42]
	1997	3	[42]
	2011	1	[42]
Poland	2002	31	[43]
Spain	2004	100	[43]
Switzerland	1983-1987	122	[7,28]
United Kingdom	1987-1989	366	[7]
	2003	255	[43]
United States of America	1983	49	[7,43]
	1985	142	[7,43]
	1998-1999	108	[7,43]
	2000	43	[7,43]
	2002	54	[7]
	2003	12	[7]
	2011	147	[39,43]
	2012	22	[39,43]
	2014	13	[39]
	2016	19	[43]
	2017	8	[43]
	2019	8	[43]

In most Asian countries, authorities and investigations are more attentive towards common illnesses like tuberculosis and dengue. A handful of evidence presently supports the likelihood of a high listeriosis prevalence [8,42,46].

In Japan, 83 predicted cases happen each year, and it is assumed that raw seafood products might contribute to those few. In China, the average recovery rate of *L. monocytogenes* was 3.7% in all food categories; raw meat was classified as the leading source [39]. In 2005, a study found a prevalence of 8.5% of *L. monocytogenes* in poultry meat in the Vidharba region of India [47]. 1.1% raw milk samples tested positive for *L. monocytogenes* collected from dairy farms across 15 major cities of Rajasthan, India, between May 2014 and November 2015 [48]. 5.21% of samples of those surfaces coming in contact with seafood on the Tuticorin coast were contaminated with *L. monocytogenes* [49]. Of all the 1615 water and raw meat samples taken from retail shops in Meghalaya and Assam, 3.9% had *L. monocytogenes* [50]. Using conventional culture and biochemical tests, Dutta et al. reported that 2% of the investigated chicken meat samples were *Listeria* positive procured from Ludhiana, India [51]. Public health officials should thoroughly inspect the outbreaks to identify contamination sources and the food vehicle. It is a disease with a high mortality rate that can be prevented by implementing correct preventive measures [7].

4. Antimicrobial Resistance (AMR) in Microorganisms: Food Sector Perspective

Antimicrobial agents are used in livestock production either for clinical treatment of infections or metaphylaxis purposes. They are also employed as growth promoters to aid animals' weight gain. In 2015, statistical data on antimicrobial consumption of various countries were collated to study its usage in flesh food animals. Each year the global usage of antimicrobials per kilogram of meat produced from cattle, chicken, and pigs were found to be averaging 45 mg, 148 mg, and 172 mg, respectively [13]. Global antibiotic consumption was predicted to escalate by 67% between 2010 and 2030. In India, antimicrobial consumption could nearly double [13]. Remarking the trends, it was gathered that middle-income countries might convert their current extensive farming practices to large-scale intensive farming; consequently, more farms would start to rely on antibiotics [52]. In both food production and medical treatment, excessive antibiotic usage led to the gradual development of resistance in pathogens towards available clinical drugs. Many countries exploit antibiotics for growth promotion in food animals to boost profits [53]. Such misuses initiate resistance due to the selection pressure on bacteria to survive. Two substantiated processes are known by which bacteria evolve resistant. Either intrinsic resistance occurs, or they acquire resistance genes [54]. Intrinsic resistance happens in evolutionary stages. Bacteria undergo a genetic mutation, and changes accumulate in the cells [55]. Acquired resistance or horizontal gene transfer typically occurs by either of the following three paths via their mobile genetic elements: [55-57].

1) Transduction-resistance genes transferred onto bacteria from another through viruses, phage related elements,

2) Conjugation-bacteria physically transfer the resistance genes to another bacteria,

3) Transformation-obtaining resistance genes from the surrounding

The emergence of bacterial antibiotic resistance was discovered around the 1980s [58]. The positive correlation between the increasing use of antibiotics and the spread of antibiotic-resistant bacteria has been documented and proved long [59,60]. These resistant bacteria transmit from animals to humans through direct contact or indirectly via the food chain and environment [61,62]. It starts with the inability of an antibiotic to eliminate resistant bacteria, which then multiply, spread and contaminate our food and environment, such as water and soil [61,62]. Eventually, reach humans via water or food, especially if raw or inadequately cooked. Cross-contamination of these microbes onto the cleaner, safer food also increases the possibility of bacterial spread [63].

5. Antimicrobial Resistance of L. monocytogenes

In 1988, the first multi-antibiotic resistant *L. monocytogenes* was identified in France [64]. Ever since, more strains have been identified as antimicrobial-resistant, particularly those isolated from food sources. Many studies stated multidrug resistance in *L. monocytogenes* strains sourced from dairy, meat, ready-to-eat foods; towards cephalosporin C, streptomycin, trimethoprim, ceftriaxone, ciprofloxacin, oxacillin, ampicillin, gentamicin, and methicillin. Initially, none of the isolates were resistant to multiple antibiotics [65]. Many of these antibiotics serve as listeriosis clinical treatment [39]. After 2006, *L. monocytogenes* strains showed borderline MIC breakpoints for ampicillin, penicillin, meropnem, and trimethoprim-sulfamethoxazole [66]. Resistance to antibiotics appears relatively common in Europe and North America [67]. Meanwhile, *L. monocytegenes* isolates in Argentina have remained susceptible to most antimicrobial agents for the past 20 years [68]. Most recovered isolates from Ganges water, milk, and human clinical samples from Varanasi, India, showed resistance to multiple antibiotics and commonly to Tetracycline [65]. S. Sharma et al.'s research proved that all collected strains from raw milk of 15 different locations in Rajasthan showed 100% resistance to four antibiotics [48].

6. Prevention and Control of Antimicrobial Resistance in Microorganisms

Each year more than four hundred thousand people succumb to food-borne illnesses, over one-third of those deaths happening within children under the age of 5years [4]. Mostly microorganisms are held responsible. When the bacteria build resistance towards available medication drugs, it becomes dreadfully complex to treat the patient, and the number of deaths occurring by food-borne diseases may increase. 'Superbug' infections cause prolonged illnesses, higher hospitalization rate, treatment failures, and ultimately death. Several pathogens have developed resistance towards most or all of the available treatments [69]. Colistin is used to treat multi-resistant bacterial infections, and owing to possible adverse effects on overall health, it should be used only when essential. However, globally doctors are now compelled to use colistin often [70]. Without effective antibiotics, major surgeries and chemotherapies are also jeopardized. Close to 10 million deaths annually can occur in India from AMR alone by 2050 [71]. Even the Indian economy would be disturbed as a result of AMR. Together with the existing high incidence of tuberculosis, malaria, typhoid, HIV in India, the lack of unerring adherence to regulations regarding the use of antibiotics in farming bring additional challenges [71].

The antimicrobial resistance issues involve various interlinked factors [72]. Cohesive action plans are required to minimize the emergence and spread of antimicrobial resistance [60,72]. WHO guidelines were developed to reduce this threat. Following 1997, World Organisation for Animal Health, Food, and Agriculture Organization (FAO) and WHO experts jointly held conferences addressing AMR in the food chain and developed WHO guidelines [73]. All countries should follow these guidelines, regardless of their region, income, or setting. They target policies, regulations, and officials overseeing animal farming matters [72,73]. All the officials and professionals connected with the flesh food production that is farm veterinarians, pharmaceutical companies, food producers, food animal organizations, public health officials, doctors, and physicians; each has a role in lowering the AMR burden. WHO developed a global action plan on antimicrobial resistance in 2015 [73]. The stated main objectives were to educate the masses and improve awareness about antimicrobial resistance, strengthen knowledge and evidence base through surveillance and research, help adopt sanitary practices and preventative steps to minimize infection incidence, optimize the available antimicrobials used for human and animal treatment, and increase investments to aid interventions, prepare new medicines, vaccines, and improve diagnostic tools [73,74]. The WHO's list of critically important antimicrobials (CIA) specifies the antibiotics crucial in our healthcare and, therefore, must be used judiciously and restricted in the veterinary sector [75,76]. The first expert meeting to prepare the CIA was held in 2005. All the participating officials meticulously considered all antimicrobial drugs and grouped them into critically important, highly important, and important. The list is periodically updated [75,76].

In India, some protocols supervise antimicrobials usage for poultry and meat food [77]. Guidelines are regularly issued on dosage criteria, tolerable levels of antibiotic residues in food products, and safer management practices. Bureau of Indian Standards (BIS) prohibited the use of growth promotion drugs in 2007, notably those with systemic effect, and additionally planned towards downscaling consumption of antibiotics targeting the gastrointestinal tract of animals within the next five years [78]. Indian department of animal husbandry, dairying, and fisheries released a circular in 2014 advising farmers to curb antibiotics and hormone addition in animal feed across the country and exercise standard-based utilization [79]. The national action plan on antimicrobial resistance (NAP-AMR) recognizes the challenges brought by the growing hazard of AMR and explains the scheme for 2017 to 2021 [80,81]. The strategies of Indian health authorities included compliance with national commitments and international collaborations, improvement of cognizance among the public through communication and extension, consolidation and enhancement of data via greater promotion and investments in research and innovation, and implementation of hygiene practices. The permissible limits of 21 antibiotics and pharmaceutically active compounds in animal meat are specified by the Food Safety and Standards Amendment of 2017; ensuring antibiotic residue limits do not threaten human health if consumed (tolerable limit of 0.01mg/kg for all meat, fats from animal sources) [82]. Although, India's efforts are not steadfast to suppress the misuse of antimicrobials in terms of growth promotion or prophylaxis [77].

Western countries are primarily the ones taking precautions to prevent the development of AMR in *L. monocytogenes* in particular. The European Union banned the blending of antibiotic additives in animal feeds from January 2006 onwards owing to the growing cases of antimicrobial-resistant *L. monocytogenes* in the environment [39]. The risks of antimicrobial-resistant *L. monocytogenes* are significantly reduced by avoiding consumption of it at all. In practice, no food industry or establishment can be clear of *Listeria* species at all times, regardless of our best efforts [44]. Tompkin suggested six steps for a Listeria control program in food processing environments [83]. They comprised of steps to prevent the entry of *Listeria* on working stations and sites, inspect the environments periodically assessing the efficiency of the program, set up rapid response procedures to correct identified contaminations, track and confirm the rectification fault by follow-up check and organize short-term assessments enabling early detection

of flaws [7,83]. Lastly, it aimed to develop long-term cyclic assessments to control contamination and to check the progress

Meat contaminates mostly between slicing and packaging operations [84]. Post-package preservation is a common technique for controlling *L. monocytogenes* in meat and its products. For decades, industrial heat sterilization was opted to produce safe foods. Non-conventional food preservation utilizes high hydrostatic pressure, ultrasound, and microwave radiation techniques. It is proved to be as efficient as thermal technologies in destroying *L. monocytogenes* and a few other food-borne pathogens [19]. Many considerations, for instance, characteristics of food components, applied sterilization technique, and microbe's ability to withstand the process, govern the efficacy of preservation processes [85-88].

Microbiological risk assessment procedures deduce factors and possibilities of an outbreak due to a pathogen in a given population. In 2003 through the collaboration of United States Food and Drug Administration (US FDA) and Food Safety and Inspection Service (USDA-FSIS), and CDC, the risk factors of listeriosis through consumption of select ready-to-eat foods were determined; as observed from three sections of people categorized by age: the fetus and newborn, elderly and general population (aged less than 60 years) [89]. Exposure models and probability assessment on consumers identified five points deciding the extent of *L. monocytogenes* exposure: amount and frequency of consumption (of the food in question; ready-to-eat (RTE) products in this case), the load of *L. monocytogenes* present on food, chances of microbial growth while storing/refrigeration of the food, temperature maintained during the refrigeration, and duration of storage/refrigeration before consumption [90-92].

Having most of the risk factors identified, the objectives of control programs become clear and focused, hence boosting the success rate [93]. A listeriosis risk assessment conducted by Food Standards Australia New Zealand (ANZFA) studied cold-smoked salmon and cooked crustaceans, noticing the rapid proliferation of the pathogen on them [94]. Therefore, stringent laws against *L. monocytegenes* presence in cooked crustaceans were implemented. Even though moderate levels of bacterial growth were observed, smoked salmon pose a significant threat, especially to the susceptible populations [7]. Based on such national risk assessments, FAO/WHO Listeria risk assessment task force adopts the evaluation procedures for RTE food products [95].

7. Conclusion

Food-borne illnesses are hindrances to good and stable public health. Bacterial infections are responsible for most of these food poisoning occurrences. As observed, *L. monocytogenes* typically contaminate poultry meat, animal meat, seafood, dairy products. Listeriosis is a mild infection in the general public. However, the severity of infection increases significantly within the high-risk population group, including the pregnant, infants, elderly, and the immune-compromised. The incidence of listeriosis is small, but the associated high mortality rate makes it concerning. Numerous researches highlighted the listeriosis hazard from meat in India. There are no prominent Indian regulations to curb *L. monocytogenes*, unlike in many western countries. Lack of proper coverage and investigation of food-borne infections lead to the absence of information on likely listeriosis outbreaks.

Meanwhile, agricultural and flesh-food sectors exploit antibiotics in their farming practices. Years of this habit led to the emergence of antimicrobial-resistant bacteria. Recent statistics indicate an increase in antibiotic usage in the future, hence raising possibilities of more and stronger antimicrobial resistance in the pathogens. Various antibiotic susceptibility test studies for *Listeria* proved multidrug resistance in many isolates. The emergence of resistant *L. monocytogenes* poses a severe health hazard. Organizations and authorities, both Indian and international, are working persistently to reduce antimicrobial resistance incidence. India's step towards controlling antimicrobial resistance is at par with other global policies and intends to lower the resistance development rate among all pathogens. Albeit, the confirmed looming listeriosis issue warrants specific codes and guidelines for the Indian scenario.

8. Conflict of Interest

The authors report no conflicts of interest.

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خطر ایجاد *لیستریا مونوسیتوژنز* مقاوم به آنتی بیوتیک در هند: مروری کوتاه و متمرکز

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چکیده

سابقه و هدف: استفاده گسترده از داروهای ضد میکروبی در بخش‌های غذایی باعث ایجاد مقاومت در میکروارگانیسم‌های موجود در محیط اطراف شد. با توجه به کاهش حساسیت نسبت به داروهای موجود، این میکروارگانیسم‌ها در هنگام درمان، میزان زنده‌مانی بیشتری دارند. بروز این مشکل در میکروارگانیسم‌های بیماریزای غذایی متداول نگران‌کننده است. چندین گونه از باکتری‌های عفونت‌زای مقاوم به ترکیبات ضدمیکروبی شناخته شده اند. *لیستریا مونوسایتوژنز* یکی از ابرمیکروب‌هایی است که چالشی برای بهداشت عمومی بحساب می‌آید. هدف این مقاله مروری بر وضعیت و موضع فعلی هند در ارتباط با مشکل روبه افزایش مقاومت به ترکیبات ضدمیکروبی و لیستریوز است.

یافته‌ها و نتیجه‌گیری: مشکل مقاومت به ترکیبات ضدمیکروبی در تمام سطوح صنایع غذایی و حوزه مراقبت‌های بهداشتی شناخته شده است. راه‌حل‌هایی دائما برای مبارزه با این مشکل درنظر گرفته می‌شود، اما به نظر نمی‌رسد که بحران مقاومت آنتی بیوتیکی را به تعویق بیندازد. علی‌رغم دادن آگاهی، وضع مقررات و محدودیت‌های جهانی، تحقیقات نشان دهنده افزایش استفاده از ترکیبات ضدمیکروبی و اطمینان عفونت‌های تهدیدکننده‌تر ناشی از آن است. گام برداشتن هند برای مهار مقاومت به ترکیبات ضدمیکروبی، همسو با سایر سیاست‌های جهانی و به منظور کاهش مقاومت تمام میکروارگانیسم‌های بیماریزا می‌باشد. تاکنون، مسئولان و مردم هند نسبت به لیستریوز سهل‌انگاری کرده‌اند. برخلاف قوانین سختگیرانه در بسیاری از کشورهای غربی، در هند قوانین خاصی با هدف‌گذاری لیستریامونوسایتوژنز وجود ندارد. دولت هند و تمام مسئولان مربوطه باید مطالعه کنند و برنامه‌های تدوین استاندارد را توسعه دهند و قوانینی برای کنترل لیستریوز وضع کنند. مهمتر از همه، راه‌اندازی سامانه مراقبت به‌منظور پایش موارد بیماری‌های مرتبط با غذا در کشور می‌باشد.

تعارض منافع: نویسندگان اعلام می‌کنند که هیچ نوع تعارض منافعی مرتبط با انتشار این مقاله ندارند.