Microbiology Spectrum
American Society for Microbiology Press

Public Health Microbiology of Shiga Toxin-Producing *Escherichia coli*

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ABSTRACT Shiga toxin-producing *Escherichia coli* (STEC) strains are the only pathogenic group of *E. coli* that has a definite zoonotic origin, with ruminants and, in particular, cattle being recognized as the major reservoir. Most human STEC infections are food borne, but the routes of transmission include direct contact with animals and a variety of environment-related exposures. Therefore, STEC public health microbiology spans the fields of medical, veterinary, food, water, and environmental microbiology, requiring a "One Health" perspective and laboratory scientists with the ability to work effectively across disciplines. Public health microbiology laboratories play a central role in the surveillance of STEC infections, as well as in the preparedness for responding to outbreaks and in providing scientific evidence for the implementation of prevention and control measures. This article reviews (i) how the integration of surveillance of STEC infections and monitoring of these pathogens in animal reservoirs and potential food vehicles may contribute to their control; (ii) the role of reference laboratories, in both the public health and veterinary and food sectors; and (iii) the public health perspectives, including those related to regulatory issues in both the European Union and the United States.

Shiga toxin-producing *Escherichia coli* (STEC) represents a major issue for public health because of the capability to cause large outbreaks and the severity of the associated illnesses (1). STEC strains are the only pathogenic group of *E. coli* that has a definite zoonotic origin, with ruminants and, in particular, cattle being recognized as the major reservoir for human infections (2). Most human infections are food borne, but the routes of transmission include direct contact with animals and a wide variety of environment-related exposures (3). Therefore, STEC public health microbiology spans the fields of medical, veterinary, food, water, and environmental microbiology, requiring a "One Health" perspective (4) and laboratory scientists with the ability to work effectively across disciplines. Public health microbiology laboratories play a central role in the surveillance of STEC infections, as well as in the preparedness for responding to outbreaks and in providing scientific evidence for the implementation of prevention and control measures. This article reviews in depth (i) how the integration of surveillance of STEC infections and monitoring of these pathogens in animal reservoirs and potential food vehicles may contribute to their control; (ii) the role of reference laboratories; and (iii) the public health perspectives, including those related to regulatory issues in both the European Union and the United States.

SURVEILLANCE AND MONITORING OF STEC INFECTIONS

Surveillance of STEC Infections in Humans

In the medical field, dedicated surveillance systems of human STEC infections have been developed in most

Received: 19 July 2013, **Accepted:** 26 July 2013,
Published: 7 November 2014

Editors: Vanessa Sperandio, University of Texas Southwestern Medical Center, Dallas, TX, and Carolyn J. Hovde, University of Idaho, Moscow, ID

Citation: Caprioli A, Scavia G, Morabito S. 2014. Public health microbiology of Shiga toxin-producing *Escherichia coli*. *Microbiol Spectrum* 2(6):EHEC-0014-2013. doi:10.1128/microbiolspec.EHEC-0014-2013.

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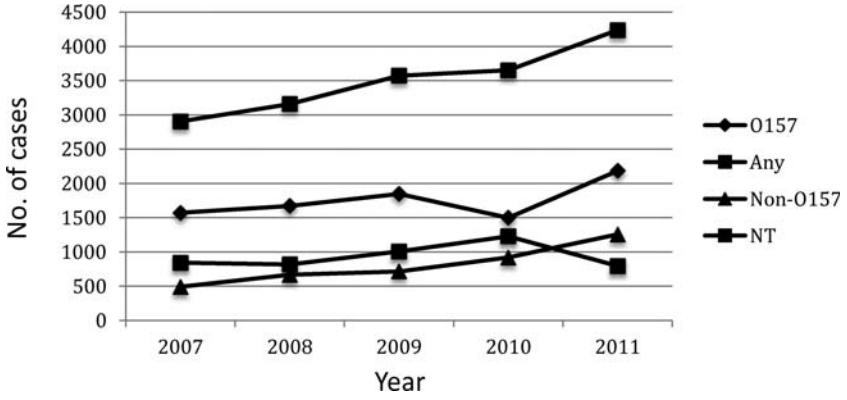
of the industrialized areas of the world because of the public health importance of these infections. Such systems are of utmost importance for the prompt recognition and management of outbreaks and the implementation of specific strategies to control the spread of the infections in the community.

Surveillance systems should record not only the epidemic outbreaks but also sporadic cases of infection. In particular, cases of severe illness, such as bloody diarrhea and the hemolytic-uremic syndrome (HUS), may reveal a wider circulation of STEC strains in a community and should be considered as possible syndromic sentinel events of possible underlying clusters of infections (5).

The data sets that should be part of surveillance systems of STEC infections include laboratory and clinical data, as well as information on risk factors and the possible association with other cases of infection. Surveillance systems are usually based on "laboratory-confirmed" case definitions that are dependent on the methods applied for laboratory diagnosis. Such methods may vary considerably. In many settings, they are limited to the detection of STEC O157, leading to an underestimation of the incidence of STEC non-O157 infections (6). The information on the clinical outcome of the reported cases of STEC infection is very important, in particular when HUS develops, since it is crucial to estimate the burden of disease due to these pathogens and to define which STEC serotypes are consistently associated with severe disease.

In Europe, the surveillance of STEC infections is embedded in the Food- and Waterborne Diseases and Zoonoses (FWD) surveillance system (http://ecdc.europa.eu/en/activities/diseaseprogrammes/fwd/Pages/index.aspx) coordinated by the European Centre for Disease Prevention and Control (ECDC). FWD is a passive surveillance system, collecting data on STEC infections from the European Union and the European Economic Area (EEA) countries, based on case definitions that include laboratory-confirmed cases, probable cases, and possible cases. For laboratory-confirmed cases, collected data include the serotype and the main virulence genes (*stx1, stx2, eae*) of the infecting strain, together with the clinical manifestation, in particular, the development of HUS. The data on STEC infections are published yearly in the *European Union Summary Reports on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks* and provide information on the trend of STEC infections in the European Union, according to the serogroup of the infecting strains and the development of HUS. Figure 1 shows the number of STEC infections reported to the ECDC-FWD surveillance system in the period 2007 to 2011 (7–11). An increasing trend of reported cases was observed, likely due to a general improvement of the surveillance systems in the participating countries. This hypothesis is supported by the sharp increase in the cases caused by STEC O157 and STEC non-O157 other than O104 notified in 2011, when the large outbreak of STEC O104:H4 infections occurred in Germany and other European countries (12). Such an increase in the reporting was likely due to the enhanced attention toward STEC infections raised by the outbreak. At the same time, a decrease in the number of cases for which the infecting strain was not serotyped was observed, probably due to the enhanced rate of submission of STEC strains to reference laboratories for confirmation and typing.

FIGURE 1 Number of STEC infections reported in the period 2007–2011 to the FWD surveillance system coordinated by the ECDC. The 2011 data do not include the cases due to STEC O104, which occurred in the framework of the large outbreak that occurred in Germany and other European countries. NT, cases with no information available on the serogroup of the STEC infecting strain. doi:10.1128/microbiolspec.EHEC-0014-2013.f1



The serogroups other than O157 and O104 most frequently associated with STEC infections in the European Union in the period 2007 to 2011 are reported in Fig. 2. STEC O26 was the serogroup most frequently reported throughout the period, followed by O103, O91, O145, and O111.

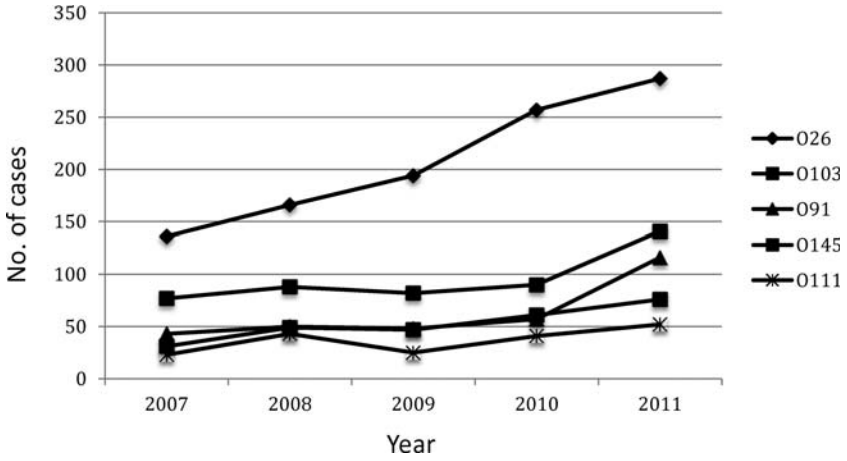
Unfortunately, not all cases reported in the ECDC-FWD databases had the complete set of data, and the information on the development of HUS was unknown for 30 to 40% of them (13). Despite this lack of information, a number of HUS cases ranging from 103 in 2007 to 277 in 2011 (not including those associated with the STEC O104 outbreak) were reported. Most cases occurred in children, with about 60% occurring in the age group 0 to 4 years. The STEC serogroups consistently associated with HUS were O157, O26, O103, O145, and O111, whereas the syndrome was rarely observed among patients with STEC O91 infection.

In the United States, the Centers for Disease Control and Prevention (CDC) established a surveillance network for cases of STEC infections and other food-borne diseases (FoodNet), with the aim of determining the incidence of laboratory-confirmed infections for bacterial pathogens transmitted commonly through food and attributing illnesses to specific sources and settings (www.cdc.gov/foodnet/). FoodNet was established in 1995 in collaboration with the U.S. Department of Agriculture's Food Safety and Inspection Service and the Food and Drug Administration. It involves 10 state health departments, with a surveillance area including 15% of the U.S. population (47 million persons). Differently from the FWD, FoodNet is an active surveillance system, with public health officials routinely communicating with the clinical laboratories to identify new cases. Cases of HUS are specifically recorded through a network of pediatric nephrologists and infection-control practitioners on the basis of clinical diagnosis (14). Infections with STEC O157 and non-O157 are included in the FoodNet surveillance system, and interestingly, similar incidence values (0.97 and 1.10 per 100,000, respectively) were recorded for the two groups in 2011 (15). However, among the patients with HUS tested with appropriate laboratory methods, the prevalence of STEC O157 infections was much higher than that of STEC non-O157. In general, among the STEC infections with the serogroup identified, the most common were O157 (47%), O26 (14%), and O103 (11%).

The FoodNet network is pulled alongside with PulseNet, a network performing standardized molecular subtyping of STEC and other food-borne pathogens by pulsed-field gel electrophoresis to detect case clusters and to facilitate the early identification of common-source outbreaks (www.cdc.gov/pulsenet/). PulseNet is also coordinated by the CDC and originated as a North American network of laboratories, then extended to other similar networks around the world (16).

Surveillance activities should be maintained over time to allow evaluating the trends of STEC infections and understanding the dynamics of the circulation of serotypes and even specific clones. In Italy, a surveillance system for HUS has been in place since 1988 (17) and has pinpointed significant changes in the prevalence of HUS-associated serogroups over time. As shown in Fig. 3, STEC O157 was associated with more than 50%

FIGURE 2 Number of STEC infections in the European Union associated with the serogroups other than O157 and O104 most frequently reported in the period 2007–2011 to the FWD surveillance system coordinated by the ECDC. doi:10.1128/microbiolspec.EHEC-0014-2013.f2



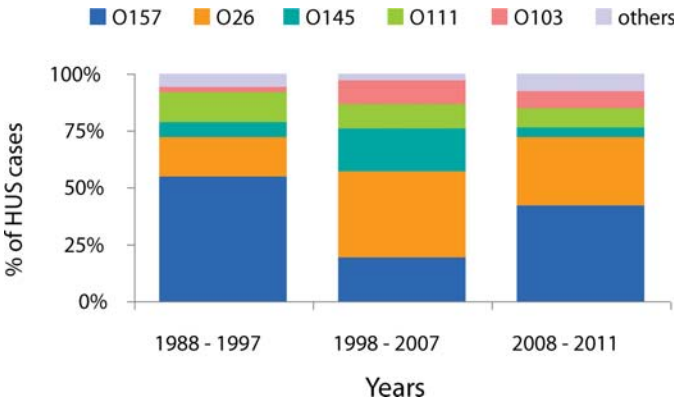


FIGURE 3 STEC serogroups associated with the hemolytic-uremic syndrome in Italy, 1988–2011. Data from the Italian Registry for HUS. doi:10.1128/microbiolspec.EHEC-0014-2013.f3

of cases in the first decade of the surveillance. After this first period, infections with STEC O26 and O145 increased, outnumbering those associated with STEC O157 in the decade 1998 to 2007. Finally, the distribution of cases due to STEC O157 and STEC O26 reached a more balanced relative figure in the current period (unpublished data from the Italian Registry for HUS, 1988–2007). Since the methods for laboratory diagnosis of STEC infection used in the surveillance (17) did not change over time, the observed variations in the prevalence of the HUS-associated serogroups likely reflect a varying exposure of the population to the sources and reservoirs of these organisms. This hypothesis might have important implications from a public health perspective and seems to be supported by the observation that patients with STEC O157 infections were older (median, 32 months) than those with non-O157 infections (median, 22 months) and showed a more clear summer peak (unpublished data from the Italian Registry for HUS, 1988–2007).

Serogroup O26 has been strongly associated with HUS also in other European countries (7–11) and the United States (18). In Europe, most of the STEC O26 pathogens causing HUS belong to a highly virulent clone, which seems to have emerged in the mid-1990s (19), with the first reports from Germany (20) and Italy (21). This O26 clone belongs to a particular multilocus sequence type, ST29, and harbors the *stx2a* subtype of Shiga toxin gene (19). It has been speculated (19) that its evolutionary success might be due to the ability to lose the *stx2a*-harboring bacteriophage (21) without entering the lytic cycle. As a matter of fact, such an ability might allow the avoidance of bacterial lysis following stimulation to release the *stx2a*-harboring bacteriophage in the human gut and might also account for a prolonged survival in the environment. Such *stx*-negative STEC O26 variants also represent targets for lysogenization by other *stx*-harboring phages, explaining their greater diffusion with respect to other lytic clones.

Monitoring of STEC Along the Food Chain

Monitoring the presence of STEC in the animal reservoirs and the potential food vehicles can provide useful information to identify which animals and foodstuffs are the main sources of human infections. However, data on the prevalence of STEC in food and animals from different investigations could be difficult to compare due to differences in the sampling strategies and analytical methods employed. As International Organization for Standardization Technical Specification (ISO/TS) 13136:2012, the international standard for the detection of STEC in food, was published only in late 2012, a variety of detection methods for STEC have been used in monitoring programs and official control plans, based on two main approaches: (i) the detection of any STEC strain present in the sample, assessed by the production of Stx and/or the presence of *stx* genes; and (ii) a serogroup-specific detection strategy, aimed in most cases at the detection of *E. coli* O157 and, more recently, a few other serogroups that have been consistently associated with HUS and are capable to cause outbreaks. These serogroups include O26, O103, O111, and O145 and have been classified in the seropathotype B group of the scheme proposed by Karmali et al. (23) (see also "A Proactive Approach to Food Control: Which STEC Should Be Considered Pathogenic?" below). In the United States, such a serogroup-specific approach has been extended to serogroups O121 and O45 that have been considered epidemiologically relevant in that country (18).

In the European Union, STEC is included among the zoonotic agents for which monitoring activities are mandatory, according to Directive 2003/99/EC, which obligates the European Union member states to collect relevant and possibly comparable data on zoonoses and zoonotic agents. Data are collected by the European Food Safety Authority (EFSA) and published in the *European Union Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne* Outbreaks (7–11) together with data on human STEC infections. EFSA issued recommendations for the monitoring of STEC in animals and food (24) that gave priority to STEC O157 as the STEC serogroup most frequently associated with severe human infections, in particular HUS, in Europe. Monitoring should then be extended to other STEC serogroups (e.g., O26, O103, O91, O145,

and O111) indicated by the periodic analysis of the data on human infections in Europe. EFSA also issued technical specifications for STEC monitoring activities (25). For animals, the sampling of the hide of young cattle and the fleeces of sheep at slaughter was indicated. For food, sampling of commodities that are perceived to be sources of STEC infections was suggested, including beef products that could be eaten with minimal cooking, ready-to-eat fermented meats, fresh produce, raw and low-heat-treated milk, and derived dairy products.

The monitoring data reported in the last published *European Union Summary Report on Zoonoses, Zoonotic Agents and Food-borne Outbreaks* (11) confirmed that STEC is mainly found in ruminants and products thereof, meat and raw milk. The proportion of STEC-positive samples can vary widely among countries, but these differences could be due to the differences in the sampling strategies and analytical methods. The reported prevalence of STEC contamination in vegetables and fruits was very low, but this probably reflects the sampling plans adopted so far; these matrices included numbers of tested samples much lower than those of foods of animal origin.

Despite the problems in the standardization of sampling and detection methods, the *European Union Summary Reports on Trends and Sources of Zoonoses, Zoonotic Agents and Food-borne Outbreaks* represent an important example of integrated medical, veterinary, and food data and can provide a valuable contribution to the source attribution of the burden of STEC infections due the serotypes and clones in humans and to the evaluation of cost-effective control measures.

The Role of Reference Laboratories

The microbiology of STEC infections is particularly complex because of the difficulties in distinguishing STEC from the other ubiquitous and generally harmless types of *E. coli*. Moreover, the physiologic and genomic ductility of these microorganisms may favor the emergence of new pathogenic clones, hindering the development of reliable schemes for their characterization. Therefore, establishing specific reference laboratories is of utmost importance for the prevention and control of STEC infections.

Reference laboratories, whether established within a normative framework or not, are usually appointed to provide reference diagnostics, reference materials, external quality assessment (EQA), scientific advice, collaboration on research and monitoring, and participation in alert and response activities. Reliable STEC reference laboratories are pivotal for supporting surveillance activities and for enabling preparedness for the threats caused by emerging pathogenic clones and epidemic outbreaks.

Public Health Reference Laboratories

Networks of STEC national reference laboratories (NRLs) have been established in the industrialized countries and operate in different contexts according to their geographic distribution.

The services provided by STEC NRLs should include confirmation, serotyping, and molecular typing of suspected *E. coli* strains isolated by front-line clinical microbiology laboratories. NRLs should also participate in surveillance activities, research, and dissemination of information and provide technical training and reference strains to front-line laboratories.

In the European Union, the public health field, differently from the food and veterinary sector, does not refer to specific norms regulating the asset of the networks of reference laboratories. Nevertheless, a web of STEC NRLs has been established over time, composed of scientific institutions historically collaborating on this topic (26) and, since 2007, connected within the framework of the ECDC-FWD program described earlier. In such a framework, the European *E. coli* NRLs provide the data on STEC infections to the ECDC-FWD database. In turn, the ECDC supports the STEC NRL network through the standardization of identification and typing methods, and the provision of reference materials (control strains) and EQA, organized to evaluate the performance of laboratories, to identify areas for improvement in laboratory methods and to ensure that identification and typing of STEC are carried out uniformly and that the results provided to the FWD database are comparable. The EQA usually includes the identification of STEC by detection and typing of the main virulence genes (*stx1*, *stx2*, *eae*) and O:H serotyping (www.ecdc.europa.eu/en/publications/_layouts/forms/Publication_DispForm.aspx?List=4f55ad51-4aed-4d32-b960-af70113dbb90&ID=1041).

In the United States, the FoodNet network supports the 650 clinical laboratories that provide data on STEC infections with laboratory protocols and recommendations and conducts periodic surveys to understand the current diagnostic practices and monitor their changes over time (27).

Veterinary/Food Reference Laboratories

The role of reference laboratories is particularly important in the veterinary/food sector. Differently from clinical microbiology, where the isolation of any STEC strain from a case of infection displaying compatible

symptoms is sufficient to establish an etiologic diagnosis, in food microbiology the need to assign a rank of risk to the STEC isolates for the humans' health does not benefit from a clear-cut definition of which STEC types have to be considered as a hazard (see "A Proactive Approach to Food Control: Which STEC Should Be Considered Pathogenic?" below). Moreover, most diagnostic tests for the detection of these organisms in food vehicles and animal reservoirs have been specifically developed for *E. coli* O157, taking advantage of its particular metabolic and antigenic features (24). Conversely, the development of assays to distinguish non-O157 STEC from nonpathogenic *E. coli* remains challenging.

In the food safety field, the duties of reference laboratories include the development, evaluation, and validation of the test methods for a reliable detection of STEC in foods, the provision of reference materials, the organization of EQA programs for the laboratories involved in food control, and the scientific and technical assistance to the public health authorities, particularly in risk assessment exercises.

In the European Union, a network of *E. coli* NRLs operates within the framework of Regulation (EC) 882/2004, which lays out the official controls on food and also establishes the nomination of European Union Reference Laboratories (EU-RLs) to face specific food and feed hazards or specific animal diseases. According to the Regulation (EC) 882/2004 prescripts, each European Union member state must designate its own NRL for each established EU-RL to create laboratory networks on the specific hazards. The NRLs collaborate with the EU-RL and coordinate the official laboratories responsible for food analyses in their country, also through the organization of national EQA. All laboratories included in the network at either the national or the European Union level must be accredited according to the norm EN ISO IEC 17025:2005. The final aim of this cascade system is that the official controls conducted on any produced or imported foodstuff are carried out using the same state-of-art methods and with comparable levels of proficiency throughout the European Union territory.

The EU-RL for *E. coli* was established in 2006, and at present it coordinates a network of 34 NRLs designated by the European Union and EEA member states (www.iss.it/vtec).

The first aim of the EU-RL was the harmonization of the identification and typing methods for STEC strains used by the veterinary/food NRLs with those used within the network of public health NRLs participating in the ECDC-FWD surveillance program to allow the comparison of data referring to STEC strains isolated from human infections and from food and animal sources. This was achieved by the organization of five EQA schemes on STEC strain identification and typing, two of which are conducted jointly with the ECDC-FWD network. The methods used in these EQA studies and the results obtained are available at the EU-RL website (www.iss.it/vtec). The results referring to the detection of the STEC main virulence genes and to the identification of the main STEC serogroups are summarized in Fig. 4 and Fig. 5, respectively.

As far as the methods for the detection of STEC in food are concerned, the EU-RL developed and published on its website several operating procedures destined to the NRL network (www.iss.it/vtec). Moreover, it coordinated the collaborative development of an international standard for the detection of these microorganisms in food upon mandate of the European Committee for Standardization. The developed standard is a horizontal method based on the real-time PCR screening of food enrichment cultures for the presence of the virulence genes (*stx1*, *stx2*, and *eae*) and genes specific for five STEC serogroups widely involved in severe human infections in Europe: O157, O26, O103, O111, and O145. Samples positive for *stx* genes are submitted to a further step aimed at isolating the STEC strain responsible for the positive PCR reactions (see also "Monitoring of STEC Along the Food Chain" above).

The standard has been published by the International Organization for Standardization in November 2012 as a technical specification (ISO/TS 13136:2012). Starting in 2009, the analytical approach described by ISO/TS 13136:2012 was evaluated in five proficiency-testing schemes organized by the EU-RL and conducted on different artificially contaminated matrices. The reports of such EQA studies are available at the EU-RL website (www.iss.it/vtec), and a synopsis of the aggregated results is shown in Fig. 6, which shows an increasing trend in the number of participating laboratories and in their analytical performances.

The existence of networks of reference laboratories reveals its pivotal value during food safety crises involving different countries, when testing food with standardized, rapid, and reliable methods is essential to provide the competent authorities the data needed to plan appropriate control measures and to inform consumers correctly. In this respect, the European Union network of *E. coli* reference laboratories was challenged in 2011 by the occurrence of the major outbreak of STEC O104:H4 infections (12). As usually occurs during food safety crises due to food-borne outbreaks,

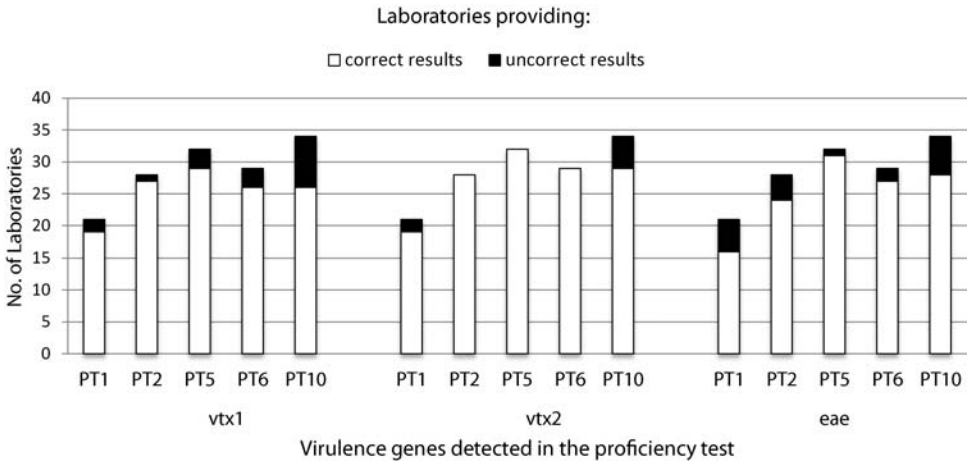
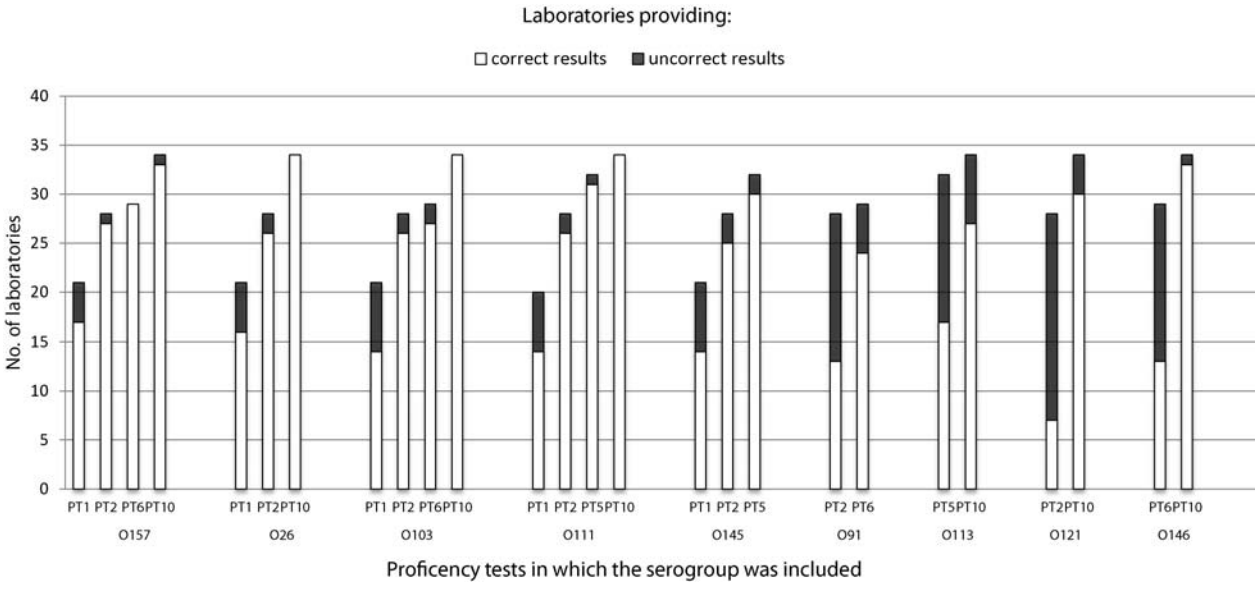


FIGURE 4 External quality assessment organized by the EU-RL for *E. coli* on the identification of STEC strains by detection of their main virulence genes by PCR. For each gene, white bars represent the number of laboratories that obtained correct results for all the strains included in the test and black bars the number of laboratories that provided incorrect results or did not perform the assay. doi:10.1128/microbiolspec.EHEC-0014-2013.f4

testing food for the presence of the outbreak strains was urgently required. Because of the activities carried out in the previous years, the European laboratory network already had a suitable screening method to exclude the presence of any type of STEC in food, tested by several rounds of EQA. An additional standard operating procedure specific for detecting the STEC O104:H4 outbreak strain was developed by the EU-RL and released through the EU-RL website 3 days after the occurrence of the outbreak was communicated (28). DNA samples to be used as positive control in the molecular assays for the detection of STEC O104:H4 were also prepared and distributed to the NRLs in the following days. During the entire period of the crisis, the EU-RL

FIGURE 5 External quality assessment organized by the EU-RL for *E. coli* on the identification of the STEC serogroups most involved in human disease in Europe. For each serogroup, white bars represent the number of laboratories that obtained correct results for all the strains included in the test and black bars the number of laboratories that provided incorrect results or did not perform the assay. doi:10.1128/microbiolspec.EHEC-0014-2013.f5



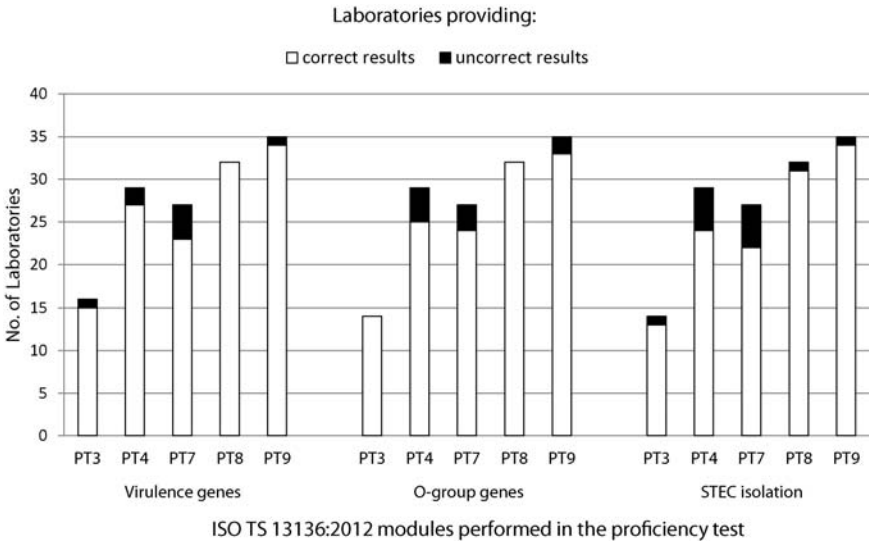


FIGURE 6 External quality assessment organized by the EU-RL for *E. coli* on the detection in food of the STEC serogroups most involved in human disease in Europe, using the real-time PCR-based ISO/TS 13136 method. For each step of the procedure, white bars represent the number of laboratories that obtained correct results for all the samples included in the test and black bars the number of laboratories that provided incorrect results or did not perform the assay. doi:10.1128/microbiolspec.EHEC-0014-2013.f6

provided continuous scientific and technical support to the European Union structures involved in the management of the crisis. The experience of the STEC O104:H4 outbreak confirmed that the activities carried out within the networks of reference laboratories can provide an important contribution in terms of preparedness to face food safety crises.

PERSPECTIVES IN PUBLIC HEALTH

A Proactive Approach to Food Control: Which STEC Should Be Considered Pathogenic?

Adequate risk assessments are crucial for securing the microbiological safety of food, and the characterization of the microbial hazards represents a milestone in such processes. A precise definition of the pathogens provides the basis for their detection and allows the assessment of their prevalence and setting the objectives for the reduction of the associated risk for human health. The characterization of STEC as a food-borne microbial hazard is complex and, at present, a definition of the characteristics associated with STEC pathogenicity remains a matter of discussion. Whether all STEC strains are pathogenic has been disputed among scientists and risk assessors for a long time; the dispute has been partially linked to the broad spectrum of symptoms associated with STEC infections, with a clinical manifestation ranging from the severe forms of HUS and hemorrhagic colitis (HC) to mild diarrhea and asymptomatic carriage. This variability in the clinical outcome, together with the STEC heterogeneity at the genomic level and the plausible effect of the general health status of the patient on the development of the disease, makes it difficult to define unambiguously the features and the genetic background of STEC that might, respectively, cause severe disease, milder symptoms, or no disease at all.

Since the 1980s, it has been proposed that the STEC strains involved in HC and HUS, also termed enterohemorrhagic *E. coli*, were restricted to particular serogroups and were characterized by the ability to induce a typical lesion in the intestine, termed "attaching and effacing" (A/E) and governed by the locus of enterocyte effacement (LEE) pathogenicity island (29). The first attempt to build a coherent classification system for pathogenic STEC was made in the early 2000s, and it is represented by the scheme developed by Karmali and coworkers (23). Such a scheme was based on the evaluation of the virulence and serological features of the strains combined with their association with severe disease and epidemic outbreaks. The integrated analysis of such data introduced the concept of "seropathotype" and led to the construction of a paradigm allocating STEC strains isolated from either human disease or the animal reservoir (Table 1).

The analysis of human cases of STEC infection notified to the ECDC-FWD surveillance system between 2007 and 2011 showed that STEC strains belonging to

serogroups O157, O26, O111, O103, and O145 were responsible for roughly 90% of the cases of HUS occurring yearly in the European Union, confirming the applicability of Karmali's scheme (7–11, 13). A similar analysis performed in the United States led to the same observation, with the exception that the most represented serogroups also included O121 and O45 (15; www.cdc.gov/foodnet). However, while the seropathotype model is effective in accommodating the strains from human infections, it presents limitations when a STEC strain isolated from a potential vehicle of infection has to be assigned to a seropathotype or has to be generally evaluated for its wherewithal of representing a hazard. As a matter of fact, STEC strains possessing the LEE pathogenicity island are commonly found in animals, the environment, or food samples, but they can belong to serotypes different from those indicated in the seropathotype scheme. Similarly, STEC strains belonging to the serotypes included in groups A and B, those allocating the most hazardous STEC strains, can possess a partial asset of virulence genes, and, theoretically, they could be considered to be less or not pathogenic at all. Finally, given the high genomic plasticity of *E. coli*, new STEC variants may emerge, with characteristics completely different from those included in the seropathotype concept (28, 30).

In spite of the limitations in categorizing the level of danger associated with STEC from nonhuman sources, the seropathotype concept raised a large consensus in the scientific community and was endorsed by EFSA, which recommended focusing food testing for STEC on the seropathotype A and B groups (24, 25). However, the massive outbreak caused by an enteroaggregative STEC O104:H4 strain in 2011 in Germany (12) has permanently questioned the efficacy of the seropathotype scheme to categorize STEC from food for the adoption of intervention measures. As a matter of fact, the STEC O104 outbreak strain was undoubtedly the most pathogenic STEC ever described and yet it did not fit the seropathotype A and B groups, being negative for the LEE and belonging to a serotype not included in the scheme (28, 31).

The crisis resulting from the STEC O104 outbreak forced the risk managers to reconsider the pathogenicity assessment for STEC, and EFSA conducted a new risk assessment exercise at the request of some European Union member states (13). A thorough evaluation of the STEC characteristics recorded in the ECDC and EFSA databases in the past 5 years led to the acknowledgment that there is not a combination of genoserotypes that identifies all pathogenic STEC strains, even when the field is narrowed to the STEC causing HC or HUS. It was recognized that some combination of virulence genes, such as particular *stx2* gene subtypes (32), together with the LEE pathogenicity island, might be associated with HUS (33, 34). However, while this observation seems to be consistent for the *stx2* subtypes, the role of the LEE in the colonization of the human intestinal mucosa could be taken over by the action of other adhesion factors, as demonstrated by the STEC O104:H4 outbreak in 2011 (28, 31). Moreover, it was highlighted that patient-associated factors, such as age, immune status, and the administration of antibiotic therapy in the early course of infection (13), have an important role in the final outcome of the STEC infection. Based on these considerations, it was recognized that neither the seropathotype concept nor the analysis of surveillance data allows a certain definition of the pathogenicity of a STEC strain or its level of danger to human health, serving as a proactive tool to protect humans' health. Such uncertainty also applies to molecular risk assessments based on the evaluation of the genetic asset only, since there are no specific genetic markers that, alone or in combination, can assign unambiguously the status of food-borne microbiological hazard to a STEC strain (13).

TABLE 1 Classification of STEC serotypes into seropathotypes[a]

Seropathotype	Relative incidence	Frequency of involvement in outbreaks[b]	Association with severe disease	Serotypes[c]
A	High	Common	Yes	O157:H7, O157:NM
B	Moderate	Uncommon	Yes	O26:H11, O103:H2, O111:NM, O121:H19, O145:NM
C	Low	Rare	Yes	O91:H21, O104:H21, O113:H21, Other
D	Low	Rare	No	Multiple
E	Nonhuman only	NA	NA	Multiple

[a]Adapted from reference 23.
[b]NA, not applicable.
[c]NM, nonmotile.

Regulatory Perspectives in the European Union and the United States

The uncertainties of STEC pathogenicity discussed above hindered the development of control measures to limit the presence of STEC in food and the issuing of specific microbiological criteria for food safety. Nevertheless, some steps toward food-safety rules have been made, often as a reaction to dramatic events such as outbreaks causing deaths and with large mass media impact. The first official measure regarding STEC was taken in the United States at the end of the 20th century. In October 1994, the U.S. Food Safety and Inspection Service declared *E. coli* O157:H7 an adulterant in ground beef in response to the multistate outbreak caused in 1993 by undercooked hamburgers contaminated with STEC O157 (35). The pronouncement was extended in 1999 to all nonintact raw beef products (36). In the 1990s, it was a popular opinion in the United States that *E. coli* O157 was the only STEC serotype causing human disease, and such a viewpoint lasted for more than 10 years. However, the growing number of infections and outbreaks caused by STEC non-O157 led to the assessment that *E. coli* O157 was not the only STEC representing a hazard (18) and the Food Safety and Inspection Service decided on the implementation of sampling and testing of beef manufacturing trimmings for STEC non-O157 starting June 4, 2012 (37). STEC strains belonging to serogroups O26, O111, O103, O145, O121, and O45 were declared as adulterant in these food commodities and included in the sampling plans in addition to *E. coli* O157. Reactions included the withdrawal from the market of the positive batches.

In the European Union, the introduction of food-safety rules related to STEC was incited by the high impact of the STEC O104:H4 outbreak (12), linked to the consumption of contaminated sprouts (38). The high number of casualties and HUS cases, together with the attention of public opinion and the backlash affecting the trade of vegetable products, forced the European Commission to take measures against the possibility that other STEC crises could occur in the European Union. EFSA was asked to perform a risk assessment exercise on the presence of STEC and other pathogenic microorganisms in sprouts and seeds intended for sprouting (38). At the same time, a technical working group, involving experts from the European Union member states and the EU-RL for *E. coli,* discussed the issues related to the definition of microbiological criteria for STEC in sprouts and the methodology to be adopted for the conformity assessment of this food commodity. The entire process took about 1 year and resulted in the issuance of Regulation (EU) 209/2013, containing the microbiological criteria for STEC in sprouts and amending Regulation (EC) 2073/2005, which lists all microbiological criteria for the assessment of the safety of food and the verification of the process hygiene criteria in the European Union. Regulation (EU) 209/2013 introduced for the first time in the European Union legislation a specific criterion for STEC regarding the presence in sprouts of the five STEC serogroups included in the seropathotypes A and B of Karmali's scheme (23) plus STEC O104:H4. One of the regulation's recitals explained that the reasons for the restriction to certain STEC groups resided in the observation that STEC O157, O26, O103, O111, and O145 are recognized as causing most of the HUS cases in the European Union, whereas STEC O104:H4 caused the large 2011 outbreak. Such a criterion might appear in opposition to the conclusions of the newly released EFSA opinion on STEC pathogenicity assessment (13) (see also "A Proactive Approach to Food Control: Which STEC Should Be Considered Pathogenic?" above). However, in agreement with such an opinion, the same regulation recital stated: "It cannot be excluded that other STEC serogroups may be pathogenic to humans as well. In fact, such STEC may cause less severe forms of disease such as diarrhoea and or bloody diarrhoea or may also cause HUS and therefore represent a hazard for the consumer's health." This last sentence widens the concept of pathogenicity to all STEC and refers to the food business operator the choice of releasing on the market sprouts positive for the presence of STEC that do not fit the microbiological criterion. This approach, apparently contradicting the role of the competent authorities in ensuring food safety, is in agreement with one of the main principles laid down in the general food law [Regulation (EC) 178/2004] that assigns to the food business operators the responsibility to place safe food on the market.

In conclusion, securing food safety is a complex matter that becomes even more complicated when dealing with STEC. As a matter of fact, STEC represents one of the most elusive pathogens in terms of phenotypic characteristics and genomic arrangement, and a continuous challenge for the laboratories in charge of assessing the food safety by applying analytical controls. Furthermore, the food market is a dynamic entity, always chasing the most available and convenient sources of food commodities capable of satisfying the need for cheap food and consumers' demand for "exotic" flavors. This often results in providers of food and raw materials from developing countries (39) introducing, from time

to time, pathogens such as the STEC O104:H4 that caused the German outbreak in 2011, bringing into question the food safety and public health systems.

Coping with such complex challenges requires the interplay of the different health care sectors. In fact, it is crucial that all roles of the risk assessment, evaluation, and management referring to both the public health and the food and veterinary fields collaborate, enforcing the concept of "One Health."

ACKNOWLEDGMENT

We declare no conflicts of interest with regard to the manuscript.

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