Enteroaggregative, Shiga toxin-producing *Escherichia coli* O104:H4 outbreak: new microbiological findings boost coordinated investigations by European public health laboratories

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In the past weeks, we witnessed the unfolding story of one of the largest ever reported outbreaks of haemolytic uremic syndrome (HUS) and bloody diarrhoea caused by Shiga toxin-producing *Escherichia coli* (STEC), also commonly referred to as verocytotoxin-producing *E. coli* (VTEC) and enterohaemorrhagic *E. coli* (EHEC) [1]. This outbreak has caused considerable suffering and resulted in a strain on healthcare and public health systems in parts in Germany. It has shown a number of striking features: an unusually large proportion of HUS cases as compared with diarrhoea cases [1]. Furthermore, whereas usually HUS triggered by STEC infection predominantly affects young children, the great majority of cases in this outbreak are adults and two thirds are women. Between 2 May and 14 June 2011, 3,332 STEC cases, including 818 cases of HUS, were reported from 13 European Union(EU)/European Economic Area (EEA) Member States and 36 patients have died [2]. Over 95% of STEC cases have been reported from Germany and the vast majority of cases reside in or have a history of recent travel to northern Germany. Additional cases related to the outbreak have been reported from Switzerland, the United States and Canada [3]. However, since 10 June, there has been a clear signal that the number of newly reported HUS and STEC cases is gradually decreasing, which suggests that we may finally be reaching the tail end of the outbreak.

The search for the source and vehicle of the outbreak has been a long and arduous process. Initial epidemiological findings pointed to raw vegetables and salads consumed in northern Germany as likely vehicles of infection and consequently led to the recommendation to abstain from eating these vegetables raw in northern Germany [1]. Extensive investigations implicated an organic sprout farm in Lower Saxony near Hamburg. Sprouts produced at this farm had been distributed

to many of the incriminated restaurants and catering facilities, and were thus identified as a likely vehicle of infection. On 10 June, German public health and food safety authorities issued a joint statement recommending people to abstain from consuming sprouts [4].

Initial laboratory analysis of clinical isolates from outbreak cases performed at the German National Reference Centre for Salmonella and other Bacterial Enteric Pathogens at the Robert Koch Institute, in Wernigerode, quickly revealed that the epidemic agent was an STEC strain of rare serotype O104:H4, with production of Shiga toxin 2 [1]. Moreover, it was further atypical in that it lacked the attaching/effacing pathogenicity island of virulent STEC strains, as indicated by negative PCR results for the intimin (*eae*) and haemolysin (*hly*) genes. All outbreak-related clinical isolates were found to be multidrug resistant and displayed indistinguishable genomic macrorestriction profiles by pulsed-field gel electrophoresis (PFGE) analysis.

In this issue of *Eurosurveillance*, a collaborative group of investigators, led by the WHO Collaborating Centre for Reference and Research on *Escherichia* and *Klebsiella*, report several intriguing and important new findings on the nature and possible origin of the epidemic strain [5]. Firstly, using well-validated genotyping methods, Scheut et al. provide convincing evidence that the STEC strain causing the outbreak in Germany is in fact not a typical virulent STEC strain, but instead is a much rarer hybrid pathotype that harbours the phage-mediated Shiga toxin determinant with an enteroaggregative *E. coli* (EAaggEC) background, more precisely described as enteroaggregative, Shiga toxin/verotoxin-producing *E. coli* (EAaggEC STEC/VTEC). Secondly, they also identify in this strain the presence of the receptor for iron-chelating aerobactin, known to be a virulence factor associated with the extra-intestinal *E.*

coli pathotype. Thirdly, they provide new data attesting to a close genetic relatedness of the German outbreak strain to previously described similar EAggEC STEC/VTEC strains. These findings are relevant for identifying the ecological reservoir and evolutionary origin of the epidemic agent, gaining a better understanding of the biological determinants of unusual disease severity and clinical complications seen in outbreak cases and the design of specific diagnostic tools for detection and treatment of STEC cases, and identification of the epidemic strain for accurate outbreak monitoring.

So what do the findings tell us about the reservoir and origin of the pathogen causing this outbreak? EAggEC is a common pathogen causing diarrhoea in travellers and persistent diarrhoea in infants and young children living in countries with poor sanitation [6,7]. In contrast to STEC strains that have an animal reservoir, mostly ruminants, EAggEC strains have a human reservoir. Little is known about the pathogenic role and epidemiological features of infections caused by strains of the hybrid EAggEC STEC/VTEC pathotype. One HUS outbreak caused by a strain of this mixed pathotype, but associated with a distinct serotype, had been previously reported from France in 1998 [8]. Scheutz et al. report that seven previously reported cases of diarrhoea or HUS worldwide caused by EAggEC O104:H4 have been identified: from Germany in 2001, France in 2004, South Korea in 2005, Georgia in 2009 and Finland in 2010 [9,10]. By PFGE analysis of EAggEC O104:H4 strains that are positive and negative for the Shiga toxin (*stx*) gene, the authors further demonstrate that, in contrast to the diversity seen within this serotype, isolates from the 2011 German outbreak cases exhibit a level of genetic similarity, which is also seen in the EAggEC STEC/VTEC O104:H4 strain from an unpublished outbreak of HUS in Georgia, which was investigated jointly by the United States Centers for Disease Control and Prevention (CDC) and Georgian public health authorities in 2009. However, no epidemiological link between these two outbreaks has been reported as yet and therefore the meaning of this finding remains elusive. Additional comparison of genomic relatedness of the German 2011 epidemic strain with other previously detected STEC O104:H4 strains causing sporadic HUS cases in other parts of the world should provide a more complete understanding of the potential reservoir and possible origin of the 2011 epidemic strain.

Another fascinating development stems from comparative genomics, available in real time, to elucidate the ancestral origin of the 2011 outbreak strain. On 2 June, further information on the nature of the hybrid EAggEC STEC/VTEC pathotype of this strain came from whole genome sequence information generated by two groups of German academic investigators [11]. Sequence information from a third isolate from a patient was subsequently generated at the Health Protection Agency, United Kingdom. The data sets from these sequencing initiatives were instantly released for public access,

resulting in data analysis among bioinformaticians and other researchers around the world. Results from these preliminary analyses have been rapidly communicated via blogs, Twitter and private web pages, outside the standard peer-reviewed scientific publication route. These initiatives have confirmed the microbiological characterisation of the outbreak strain made in the public health laboratories by targeted genotyping and phenotyping of facultative *E. coli* virulence genes. Most importantly, among compared *E. coli* genome sequences, the genome of the 2011 outbreak strain clustered closest to an EAggEC strain isolated in 2002, with the addition of *stx2* and antibiotic resistance genes.

How do these microbiological findings help clinical and public health laboratories detect and confirm cases in a timely and reliable manner? Further to key information provided by the Robert Koch Institute on strain screening and characterisation, Scheutz et al. also propose an alternative simple laboratory screening tool for detecting the 2011 German outbreak strain: a bacterial cell slide agglutination assay with cross-reacting antiserum against the capsular K9 antigen. This test, depending on reagent availability, can be used for the primary laboratory detection of *E. coli* O104:H4 in faecal specimens from suspected cases. Therefore, this assay enhances the potential capability of microbiology laboratories to detect and report cases accurately to clinical practitioners treating the patients and to public health authorities investigating the outbreak.

In summary, from a scientific perspective, the major findings reported in this issue by Scheutz et al. shed light on the unusual pathogenic features, prior occurrence in human pathology and likely natural reservoir of the *E. coli* strain causing the ongoing HUS and diarrhoea outbreak in Germany. More studies are needed to understand which and how these biological features of the bacterium actually determined the unique clinical and epidemiological disease manifestations in this outbreak.

Furthermore, from a public health perspective, it should be emphasised that the microbiology findings and technical recommendation presented were immediately shared by the authors through EU and international public health and food safety laboratory alert networks. This timely dissemination of key data to those who need to know has included posting technical information on the European Centre for Disease Prevention and Control (ECDC)-supported Epidemic Intelligence Information System (EPIS) rapid exchange platform. The EPIS links together all EU/EEA public health laboratories in the Food- and Waterborne Diseases and Zoonoses network (FWD-Net). In parallel, the European Union Reference Laboratory for Verotoxin-producing *E. coli* rapidly developed a real-time PCR method to detect O104 somatic- and H4 flagellar antigen-associated genes in food samples and shared it with the EU veterinary and food safety reference laboratory network.

This approach illustrates how seamless collaboration between food and public health laboratories, as well the power of harnessing advanced molecular typing technology and electronic communication, can build the laboratory capacity needed to respond appropriately to the cross-border spread of a highly virulent food-borne pathogen.

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