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Molecular epidemiological view on Shiga toxin-producing *Escherichia coli* causing human disease in Germany: Diversity, prevalence, and outbreaks

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ABSTRACT

Infections by intestinal pathogenic *Escherichia coli* (*E. coli*) are among those causing a high mortality and morbidity due to diarrheal disease and post infection sequelae worldwide. Since introduction of the Infection Protection Act in Germany 2001, these pathogens rank third among bacterial infections of the gastrointestinal tract. As a major pathovar Shiga toxin-producing *E. coli* (STEC) which include enterohemorrhagic *E. coli* (EHEC) play a leading role in occurrence of sporadic cases and disease outbreaks. An outstanding example is the large outbreak in spring 2011 caused by EHEC/EAEC O104:H4. To monitor and trace back STEC infections, national surveillance programs have been implemented including activities of the German National Reference Centre for *Salmonella* and other Enteric Bacterial Pathogens (NRC). This review highlights advances in our understanding of STEC in the last 20 years of STEC surveillance by the NRC. Here important characteristics of STEC strains from human infections and outbreaks in Germany between 1997 and 2013 are summarized.

1. Introduction

The German National Reference Centre for *Salmonella* and other Enteric Bacterial Pathogens (NRC) hosted by the Robert Koch Institute Division of Enteropathogenic Bacteria and *Legionella* has been surveilling important bacterial diarrheal pathogens causing disease in humans since 1993 (Homepage: http://www.rki.de/DE/Content/Infekt/NRZ/Salmonellen/salmo_node.html;jsessionid=1D05B5E97ACCB8543EF4E41721E3E0E4.2_cid381). Our intention is to obtain an overview about the strain types and their prevalence, virulence, antibiotic resistance, and persistence. Further, changes in the pathogen types are recognized which may be indicative of new emerging or disappearing strain variants and may give a hint on outbreaks. Outbreak detection is performed in cooperation with the department of infection epidemiology of the Robert Koch Institute and other partners, such as the Consultant Laboratory for Hemolytic Uremic Syndrome (HUS) at the University of Münster, the Public Health Authorities of the German Federal States and the Federal Institute of Risk Assessment (BfR). Upon strain comparison and by means of specific strain properties also infection sources can be traced together with other public health, food or veterinary authorities. The NRC further is involved in the development and improvement of diagnostic procedures.

Within the 20 years of the work of the NRC, more than 12,000 Shiga toxin-producing *E. coli* (STEC) isolates were collected and thoroughly analyzed yielding in a comprehensively studied strain collection. The term STEC is used throughout this article for strains of both, typical enterohemorrhagic *E. coli* (EHEC) serovars which are highly associated with sequelae like hemorrhagic colitis (HC) and HUS, involving thrombocytopenia, haemolytic anemia, and acute renal failure, and many other serovars which are only rarely associated with severe human disease. With respect to intestinal pathogenic *E. coli* microbiology, pathogenicity, clinical issues, and evolution, the reader is referred to a recently published comprehensive review (Croxen et al., 2013) and therefore, we here will concentrate on EHEC surveillance at NRC. Within the period of STEC surveillance, the strains analyzed at NRC represent about 60–80% of the reported cases in Germany ranging from mild or bloody diarrhea up to HUS. To the purpose of strain characterization, bacterial isolates are mainly sent from public health authorities and in some cases from primary diagnostic laboratories to the NRC for subtyping. At the NRC, a variety of classical phenotypic as well as DNA-based methods are used.

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In the case of STEC, strain isolation is currently rarely performed in primary diagnostic laboratories. The procedure is very labour intensive and in most cases the determination of the presence of the Shiga toxin or its genes from stool samples or enrichment cultures is sufficient for therapeutic decisions and case reporting according to the German Infection Protection Act. Therefore, isolation of the pathogen and further subtyping is usually performed in specialized or reference laboratories. Phenotypic and DNA-based subtyping is performed on STEC isolates for *E. coli* pathovar determination and for clone differentiation for epidemiological purposes. A broad spectrum of established mostly PCR-based methods for the detection of virulence determinants, such as adhesin or toxin genes and their variants, is employed for pathovar characterization (see Table 1; Flieger et al., 2013). These PCR-based detections of gene regions are important methods for epidemiological analysis of STEC however cannot evaluate functionality of gene expression or of the corresponding proteins since non-functional degenerated gene mutants have been described (Abu-Ali et al., 2010). Strain subtyping by means of serotyping either via classical or genoserotyping and by biochemotyping, such as sorbitol fermentation, is carried out for all STEC isolates sent to the NRC. Further, international standardized pulsed-field gel electrophoresis (PFGE) after DNA macrorestriction is still the method of choice for reliable identification of outbreak-associated STEC clones. Serotyping, PCR-based virulence gene profiling, and PFGE analysis were important tools applied during the 2011 EHEC/EAEC O104:H4 outbreak for characterization of the outbreak strain and for tracing of infection clusters (Bielaszewska et al., 2011; Frank et al., 2011; Sin et al., 2013; Robert Koch Institute, 2011).

In this review, we present a summary of important characteristics of STEC isolated from human infections in Germany between 1997 and 2013. Used abbreviations and gene names with respect to STEC subtyping are explained and referenced in Table 1.

2. STEC O serogroups causing human disease

Serotyping is an important method for STEC subtyping. At the NRC classical serotyping is performed as described by Ørskov and Ørskov (1984). So far 186 O serogroups (O1-O192 except O31, O47, O67, O72, O93 and O94) and 53 associated H forms (H1-56 except H13, H22, H50) are currently described (see WHO Center for *Klebsiella* and *Escherichia coli*, Copenhagen, Denmark for further information). Within the here reported period, 139 different O serogroups were detected among STEC strains. Most commonly found O serogroups among STEC were O157, O91, O26 and O103 which represent about 40 to 50% of the STEC strains analyzed at NRC starting from 1999 (Fig. 1). Excluding 2011, this shows that there are no major changes in O serogroup distribution and consequently in their significance in human disease. It is also obvious that the percentage of O157 strains decreased from about 25% in 1999 to about 10% in 2013. This may reflect the growing awareness of other STEC serogroups in diagnostics rather than a significant decrease in O157 infections. Accordingly, the fraction of O91 strains increased from about 5% in 1999 to about 15% in 2012 and 2013. The large outbreak due to EHEC/EAEC O104:H4 in 2011 highly influenced O serogroup distribution by representing about 50% of the strains analyzed in that year. Most frequent H type combinations of the most important O serogroups were O157:Hnm/H7, O91:Hnm/H14, O26:Hnm/H11, and O103:H2 (Table 2). It is further interesting to note that about 10% of the strains yearly are either untypable or cannot be attributed to a known O serogroup which shows a limitation of classical serotyping compared to DNA-based methods addressing coding genes for expression of serotype-related traits.

In summary, O26, O91, O103, and O157 were the most common O serogroups occurring among STEC from 1997 to 2013 and only slight changes in their distribution can be observed. Except in 2011, the large outbreak due to EHEC/EAEC O104:H4 dominated O serogroup percentages (Fig. 1).

Table 2
Most frequently observed combinations of O and H antigens among human STEC isolates between 1997 and 2013 (NRC collection).

O-antigen	H-antigen most frequently observed	Other H antigens
5	nm	11/40
8	nm/19	4/8/10/14/21/25/28/49
26	nm/11	21
55	nm/7	6/12/31
76	nm/19	7/nt
80	2	nm/21/32
91	nm/14	21
103	2	nm/11/18
104	unknown	nm/4/7/21
111	nm	8
113	nm/4	6/11/21/28
115	10	nm/8
118	16	nm/12
128	2	nm/2/10
145	nm	25/28/34
146	21/28	nm/8/10/31
156	25	nm/1/7
157	nm/7	–
174	2/8	nm/21/24/28/35
177	nm/11	45

Table 1
Gene names and abbreviations used.

Genes/abbreviations	Descriptions	Reference
stx (*stx*$_{1a,1c,1d,2a,2b,2c,2d,2e,2f,g}$)	Shiga toxin gene(s)	Scheutz et al. (2012)
*agg*R, *aat*A, *agg*3A, *agg*3C, *agg*A, *agg*C, *hda*	Genes associated with aggregative adhesion	Boisen et al. (2008)
aaiC	Type six secretion substrate gene	Dudley et al. (2006)
*est*Ia	Heat-stable enterotoxin gene	Nada et al. (2013)
eaeA	Intimin gene	Schmidt et al. (1993)
fliC	Flagellin gene	Kuwajima et al. (1986)
saa	Autoagglutinating adhesin gene	Paton et al. (2001)
*eib*G	Immunoglobulin-binding protein gene	Lu et al. (2006)
*sfp*A	Major subunit of Sfp fimbriae gene	Friedrich et al. (2004)
sub *toxB*	Subtilase cytotoxin gene Virulence gene involved in adhesion located on pO157 plasmid	Paton and Paton (2005)Toma et al. (2004)
O-antigen	Surface antigen	Ørskov and Ørskov (1984)
H-antigen	Flagellar antigen	Ørskov and Ørskov (1984)
nm	Non motile	–
nt	Not typeable	–
PT	Phage type	–
sf/nsf	Sorbitol fermenting/ non-sorbitol fermenting	–