BfR, NIFDS, ANSES, DTU
Joint International Symposium
„Global past, present and future challenges in risk assessment - Strengthening Consumer Health Protection"

How have Germany and France responded to the crisis with Enterohemorrhagic *E. coli* O104:H4 and what about the future?

Patrick FACH

The Agency for Food, Environmental and Occupational Health & Safety (Anses)

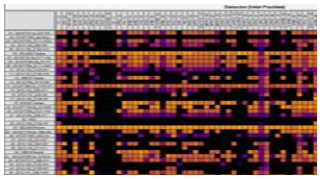
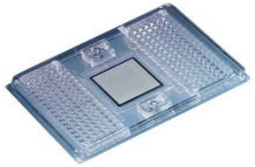
14, rue Pierre et Marie curie,
94701 Maisons-Alfort, France

30th November -1st December 2017, BfR, Berlin, Germany

The IdentyPath Genomic Platform of Anses

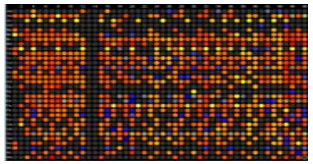
BioMark (Fluidigm)

Gene detection & expression, SNP genotyping,
Absolute quantification = Digital PCR

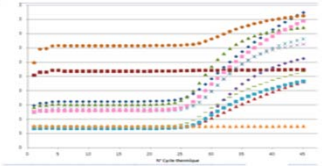
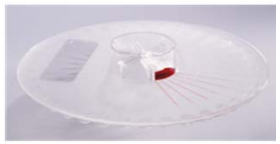
Light Cycler 1536 (Roche)

Gene detection & expression, SNP genotyping,
Absolute quantification = Digital PCR


GeneDisc Cycler (Pall)

Gene detection & expression

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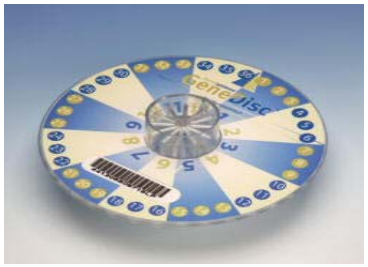

PhD student shared by Anses and BfR (2010-2012)

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Micro-array for the identification of Shiga toxin-producing *Escherichia coli* (STEC) seropathotypes associated with Hemorrhagic Colitis and Hemolytic Uremic Syndrome in humans

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BfR Anses

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ABSTRACT

A micro-array has been developed, based on the GeneDisc® array, for the genetic identification of 12 O-types and 7 H-types of Shiga toxin-producing *Escherichia coli* (STEC) including the most clinically relevant enterohemorrhagic *E. coli* (EHEC) serotypes. The genes selected for determination of the O antigens ($rfbE_{O157}$, wzx_{O26}, wzx_{O103}, $wbd1_{O111}$, $ihp1_{O145}$, wzx_{O121}, wzy_{O113}, wzy_{O91}, wzx_{O104}, wzy_{O118}, wzx_{O45}, and $wbgN_{O55}$) and H-types ($fliC_{H2}$, $fliC_{H7}$, $fliC_{H8}$, $fliG_{H11}$, $fliG_{H19}$, $fliC_{H21}$, and $fliG_{H28}$) showed a high specificity and concordance with serology. The micro-array also had a high specificity for EHEC-associated virulence factors, including Shiga toxins 1 and 2 (*stx*1 and *stx*2), intimin (*eae*), enterohemolysin (*ehxA*), serine protease (*espP*), catalase peroxidase (*katP*), the type II secretion system (*etpD*), subtilase cytotoxin (*subA*), autoagglutinating adhesin (*Saa*) and type III secreted effectors encoded in the genomic islands OI-122 (*ent/espL2*, *nleB*, and *nleE*) and OI-71 (*nleF*, *nleH1-2*, and *nleA*). The *eae* gene was detected in all typical EHEC strains, and the pattern of *nle* genes encoded in OI-71 and OI-122 was found to be closely associated with certain serotypes of typical EHEC and emerging EHEC strains. Virulence plasmid associated genes such as *katP*, *espP*, and *etpD* were more common in EHEC than in STEC strains; this supports their association with virulence. This array constitutes a valuable approach for the identification of STEC strains with a high potential for human virulence.

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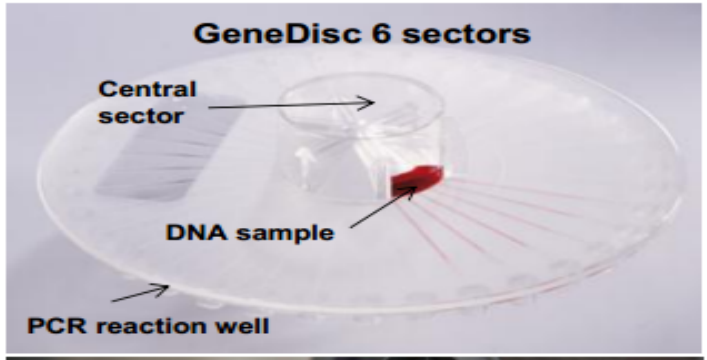
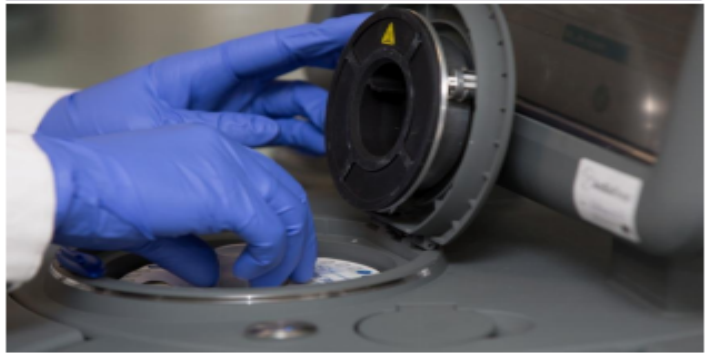
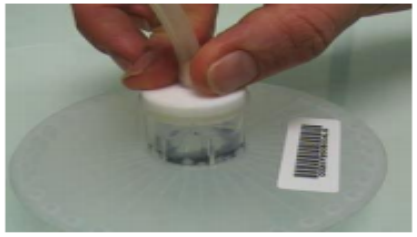
E. coli O104:H4 qPCR detection

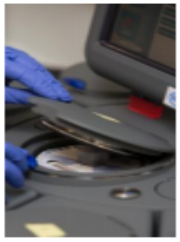
GeneDisc Cycler (Pall)

Gene detection, gene expression









- Discs with 6, 9 or12 sectors
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- Multiplexing
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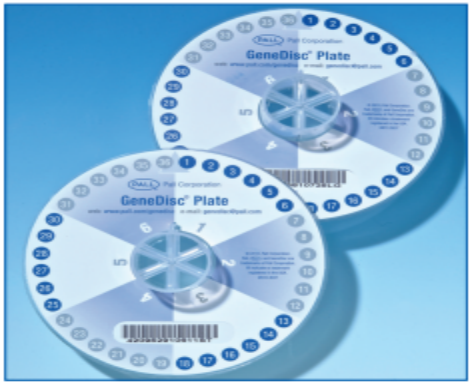
E. coli O104:H4 detection

Data Sheet FBDSGDECO104EN

GeneDisc® Technologies

For an easy, rapid and specific detection of pathogenic *E. coli* O104:H4 in food

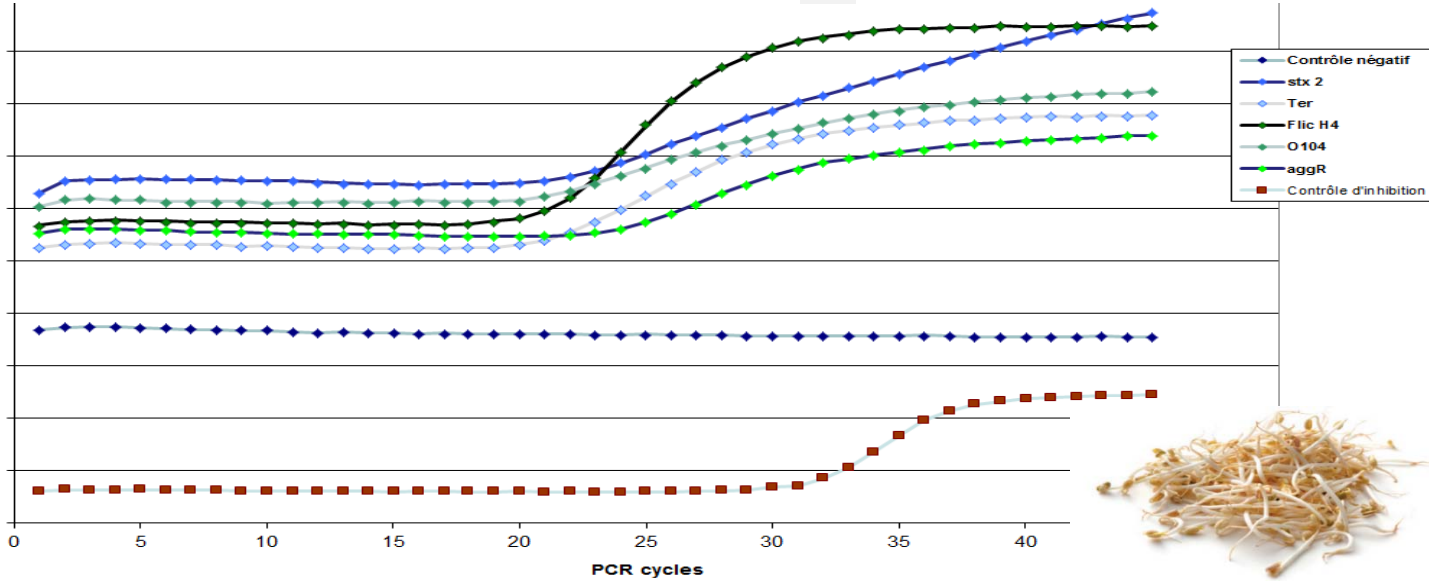


E. coli O104:H4 2011 Outbreak Strain ID

Bacteria	Gram –, motile enterobacteria
Genetic markers of 2011 crisis strain	• Shigatoxin *stx2* • *terE* (tellurite resistance gene cluster) • *aggR* (master regulator of virulence genes) • Multi-resistance pattern to antimicrobials
Disease	Gastro-enteritis, Hemorrhagic diarrhea, Hemolytic-uremic syndrome (HUS)
Source of contamination	Fenugreek seeds

Developement of a multiparametric PCR targeting

stx2, rfb_{O104}, $fliC_{H4}$, terD & aggR.



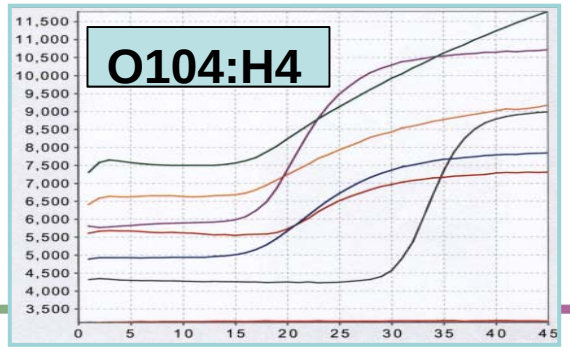
Based on the common work conducted at the **BfR** and **ANSES** this qPCR has been made commercially available within few weeks after the beginning of the crisis

Outbreak investigations at the NRL for E. coli (BfR)

668 food and environmental samples were investigated (30. May - 13. July)

(pictures from Lothar Beutin, BfR)

Vegetables, water, environmental samples, hundreds of sprout and seed samples



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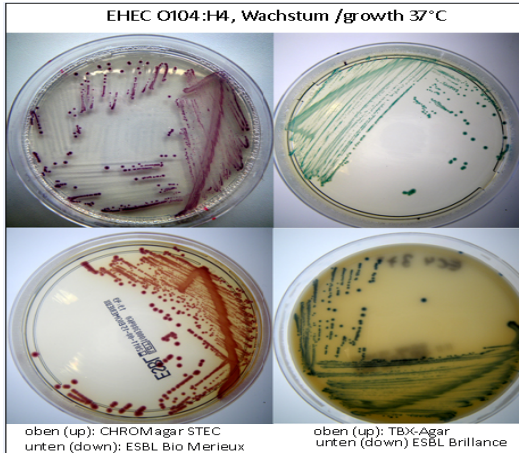
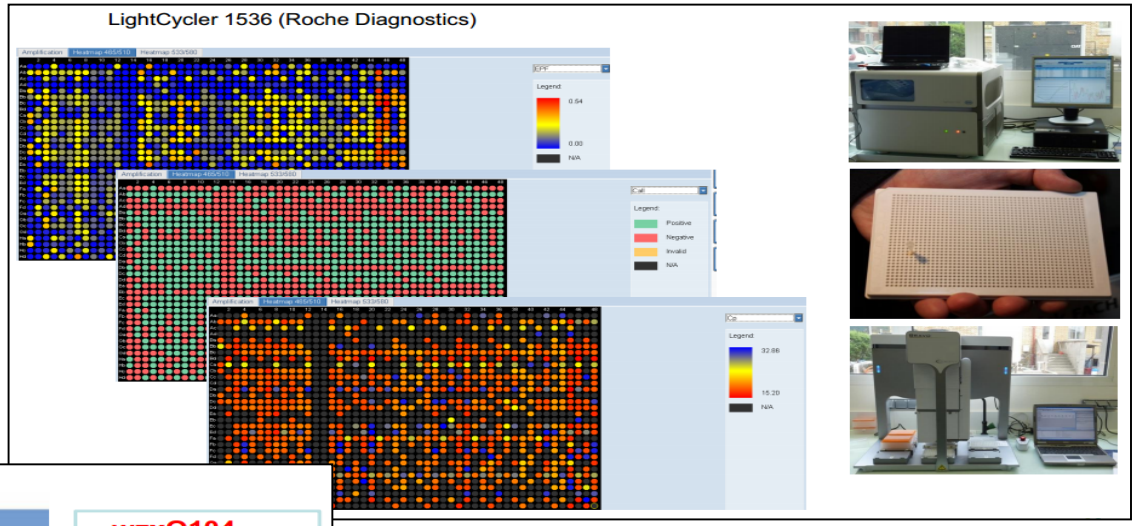
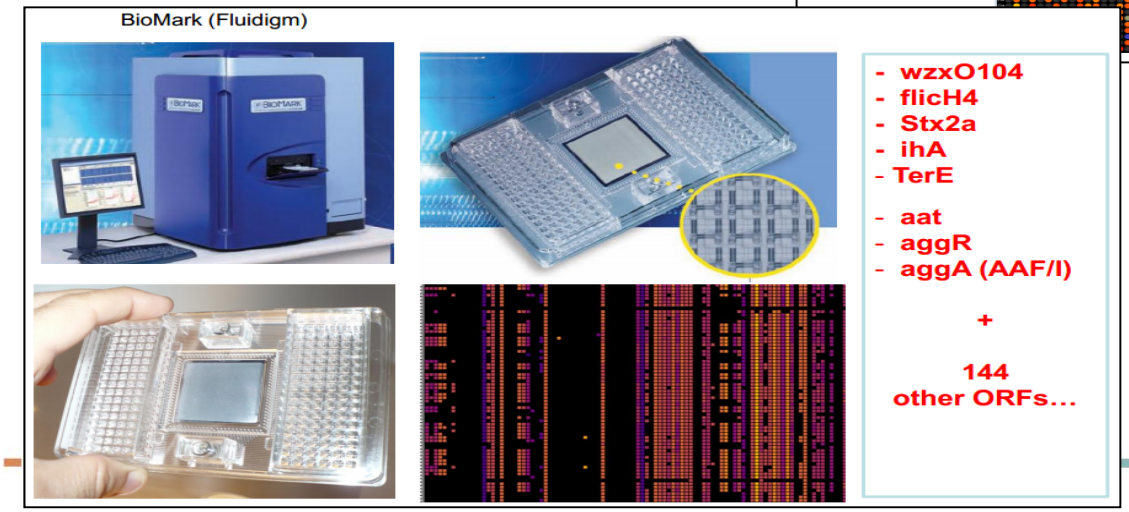
Characterization of *E. coli* O104:H4 isolates







High throughput qPCR :

qPCR Microarray

qPCR on chips

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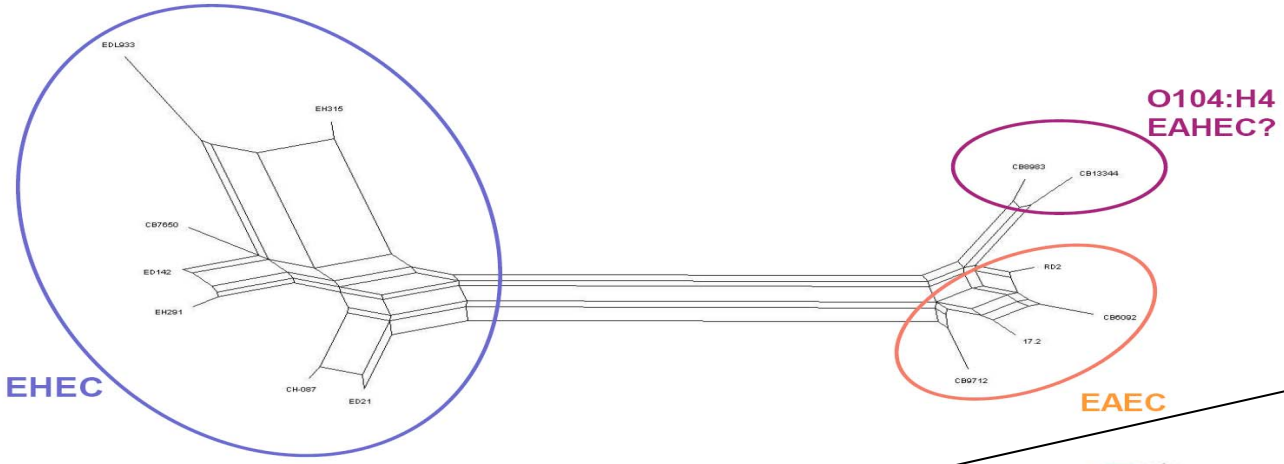
Characterization of *E. coli* O104:H4 by high throughput qPCR

		O104:H4 _ 2011 CB13344	O104:H4 _ 2001 CB8983
stx1	Shiga toxin 1	-	-
stx2	Shiga toxin 2	+	+
vtx2a	Shiga toxin 2a	+	+
wzx $_{O104}$	O104 somatic antigen	+	+
fliC $_{H4}$	H4 flagellar antigen	+	+
aggA	Aggregative adherence fimbriae I (AAF/I)	+	-
agg3A	Aggregative adherence fimbriae III (AAF/III)	-	+
aap	Dispersin	+	+
aatA	ABC-transporter protein (pAA) (pCVD432)	+	+
irp2	Component of iron uptake system on HPI	+	+
fyuA	Component of iron uptake system on HPI	+	+
pic	Pic (protein involved in intestinal colonization)	+	+
set1	*Shigella* enterotoxin 1	+	+
aggR	Transcriptional regulator AggR	+	+
astA	EAEC heat-stable enterotoxin 1 (EAST1)	-	+
iha	Iha (IrgA homolog adhesin)	+	+
lpfA $_{O113}$	Structural subunit of long polar fimbriae (LPF) of STEC O113	+	+
lpfA $_{O26}$	Structural subunit of long polar fimbriae (LPF) of STEC O26	+	+
lpfA $_{O157}$	Structural subunit of long polar fimbriae (LPF) of STEC O157	-	-
bfpA	Bundle-forming pili	-	-
sfpA	Structural subunit of Sfp fimbriae	-	-
bla $_{CTX-M15}$	Beta-lactams resistance	+	-
bla $_{TEM-1}$	Beta-lactams resistance	+	+
terE	Tellurite resistance	+	+
ureD	Urease UreD	-	-
ehxA	EHEC entero-haemolysin	-	-
espP	Serine protease EspP	-	-
etpD	Type II secretion system	-	-
katP	Catalase / Peroxydase	-	-
saa	Saa (STEC autoagglutinating adhesin)	-	-
subA	Subtilase cytotoxin	-	-
toxB	Putative EHEC adhesin	-	-

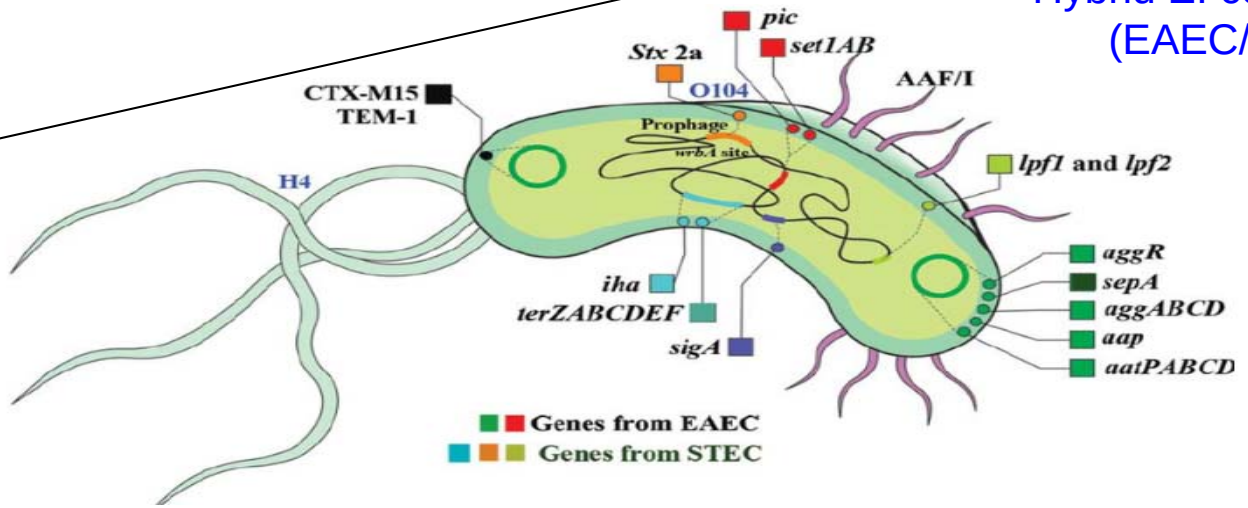
Characterization of *E. coli* O104:H4 by high throughput qPCR

		O104:H4 _ 2011 CB13344	O104:H4 _ 2001 CB8983
eae *	All the ORFs of the LEE have been tested (41 ORFs)	-	-
pagC	Type III secreted effector encoded in the genomic islands OI-122	-	-
efa1	Type III secreted effector encoded in the genomic islands OI-122	-	-
efa2	Type III secreted effector encoded in the genomic islands OI-122	-	-
ent/espL2	Type III secreted effector encoded in the genomic islands OI-122	-	-
nleB	Type III secreted effector encoded in the genomic islands OI-122	-	-
nleE	Type III secreted effector encoded in the genomic islands OI-122	-	-
espO1-1	Type III secreted effector protein	-	-
espK	Type III secreted effector protein	-	-
espM1	Type III secreted effector protein	-	-
espM2	Type III secreted effector protein	-	-
espR1	Type III secreted effector protein	+	+
espV	Type III secreted effector protein	-	-
espW	Type III secreted effector protein	-	-
espX1	Type III secreted effector protein	+	+
espX2	Type III secreted effector protein	-	-
espX5	Type III secreted effector protein	+	+
espX6	Type III secreted effector protein	-	-
espX7	Type III secreted effector protein	-	-
espY3	Type III secreted effector protein	-	-
espY4	Type III secreted effector protein	-	-
nleC	Type III secreted effector protein	-	-
nleD	Type III secreted effector protein	-	-
nleF	Type III secreted effector protein	-	-
nleG	Type III secreted effector protein	-	-
nleG6-2	Type III secreted effector protein	-	-
nleG8-2	Type III secreted effector protein	-	-
nleH1-1	Type III secreted effector protein	-	-
efa2	Type III secreted effector protein	-	-
espN	Type III secreted effector protein	-	-
nleG2	Type III secreted effector protein	-	-

Characterization of *E. coli* O104:H4 by high throughput qPCR



Hybrid E. coli O104:H4 (EAEC/STEC).



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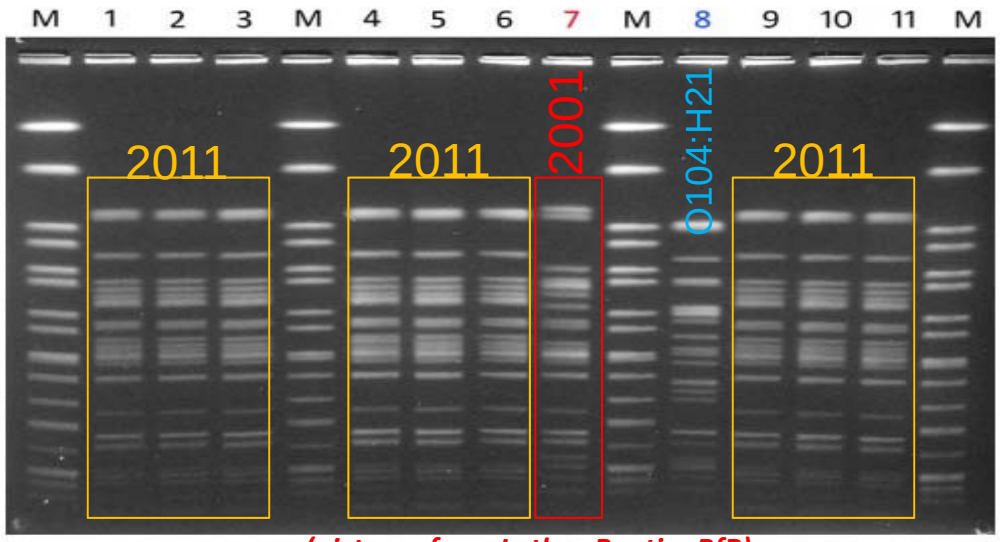
PFGE profile (XbaI) of human and non-human O104:H4 isolates

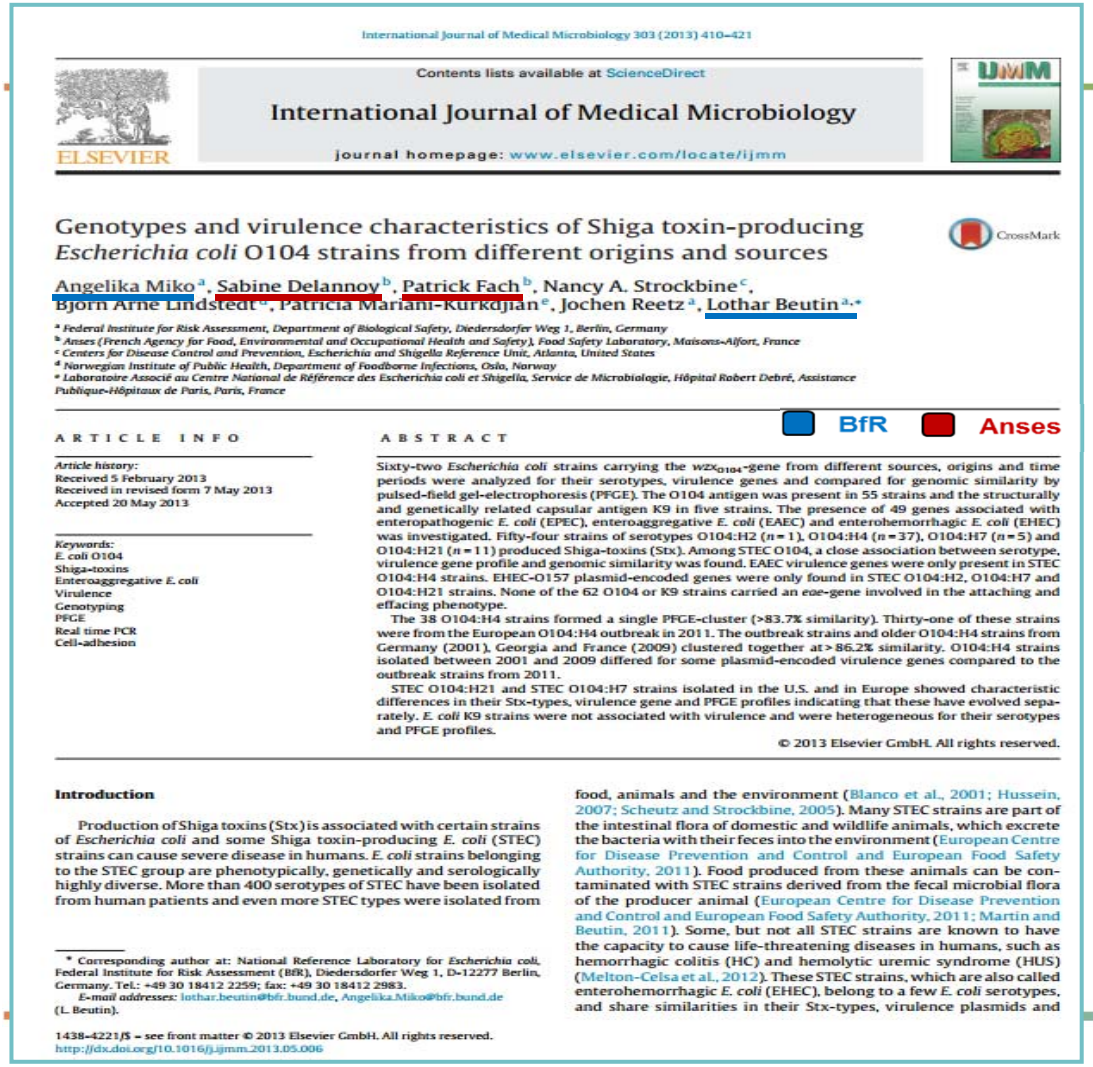


(pictures from Lothar Beutin, BfR)

Track	serotype	source, date	origin
1	O104:H4	HUS, May 2011	RKI, index strain
2	O104:H4	HUS May.2011	Berlin
3	O104:H4	HUS, May 2011	Cologne
4	O104:H4	HUS, May 2011	Magdeburg
5	O104:H4	Cucumber, May 2011	
6	O104:H4	sprouts, May 2011	intital source sprout farm Bienenbüttel
7	O104:H4	HUS, September 2001	Cologne
8	O104:H21	Meat, November 2005	Erlangen,
9	O104:H4	Paprika, June 2011t	Catering, Kaufungen
10	O104:H4	smoked salmon, June 2011	
11	O104:H4	cooked salmon, June 2011	

All O104:H4 strains from the May/June 2011 outbreak show the same PFGE profiles

The profiles of the first O104:H4 isolate in 2001 (Lane 7) differs from the 2011 outbreak strain, as does an O104:H21 isolate (Lane 8)



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Genotypes and virulence characteristics of Shiga toxin-producing *Escherichia coli* O104 strains from different origins and sources

Angelika Miko[a], Sabine Delannoy[b], Patrick Fach[b], Nancy A. Strockbine[c], Björn Arne Lindstedt[d], Patricia Mariani-Kurkdjian[e], Jochen Reetz[a], Lothar Beutin[a,*]

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Cell-adhesion

ABSTRACT

Sixty-two *Escherichia coli* strains carrying the wzx_{O104}-gene from different sources, origins and time periods were analyzed for their serotypes, virulence genes and compared for genomic similarity by pulsed-field gel-electrophoresis (PFGE). The O104 antigen was present in 55 strains and the structurally and genetically related capsular antigen K9 in five strains. The presence of 49 genes associated with enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC) and enterohemorrhagic *E. coli* (EHEC) was investigated. Fifty-four strains of serotypes O104:H2 ($n = 1$), O104:H4 ($n = 37$), O104:H7 ($n = 5$) and O104:H21 ($n = 11$) produced Shiga-toxins (Stx). Among STEC O104, a close association between serotype, virulence gene profile and genomic similarity was found. EAEC virulence genes were only present in STEC O104:H4 strains. EHEC-O157 plasmid-encoded genes were only found in STEC O104:H2, O104:H7 and O104:H21 strains. None of the 62 O104 or K9 strains carried an *eae*-gene involved in the attaching and effacing phenotype.

The 38 O104:H4 strains formed a single PFGE-cluster (>83.7% similarity). Thirty-one of these strains were from the European O104:H4 outbreak in 2011. The outbreak strains and older O104:H4 strains from Germany (2001), Georgia and France (2009) clustered together at > 86.2% similarity. O104:H4 strains isolated between 2001 and 2009 differed for some plasmid-encoded virulence genes compared to the outbreak strains from 2011.

STEC O104:H21 and STEC O104:H7 strains isolated in the U.S. and in Europe showed characteristic differences in their Stx-types, virulence gene and PFGE profiles indicating that these have evolved separately. *E. coli* K9 strains were not associated with virulence and were heterogeneous for their serotypes and PFGE profiles.

Introduction

Production of Shiga toxins (Stx) is associated with certain strains of *Escherichia coli* and some Shiga toxin-producing *E. coli* (STEC) strains can cause severe disease in humans. *E. coli* strains belonging to the STEC group are phenotypically, genetically and serologically highly diverse. More than 400 serotypes of STEC have been isolated from human patients and even more STEC types were isolated from food, animals and the environment (Blanco et al., 2001; Hussein, 2007; Scheutz and Strockbine, 2005). Many STEC strains are part of the intestinal flora of domestic and wildlife animals, which excrete the bacteria with their feces into the environment (European Centre for Disease Prevention and Control and European Food Safety Authority, 2011). Food produced from these animals can be contaminated with STEC strains derived from the fecal microbial flora of the producer animal (European Centre for Disease Prevention and Control and European Food Safety Authority, 2011; Martin and Beutin, 2011). Some, but not all STEC strains are known to have the capacity to cause life-threatening diseases in humans, such as hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS) (Melton-Celsa et al., 2012). These STEC strains, which are also called enterohemorrhagic *E. coli* (EHEC), belong to a few *E. coli* serotypes, and share similarities in their Stx-types, virulence plasmids and

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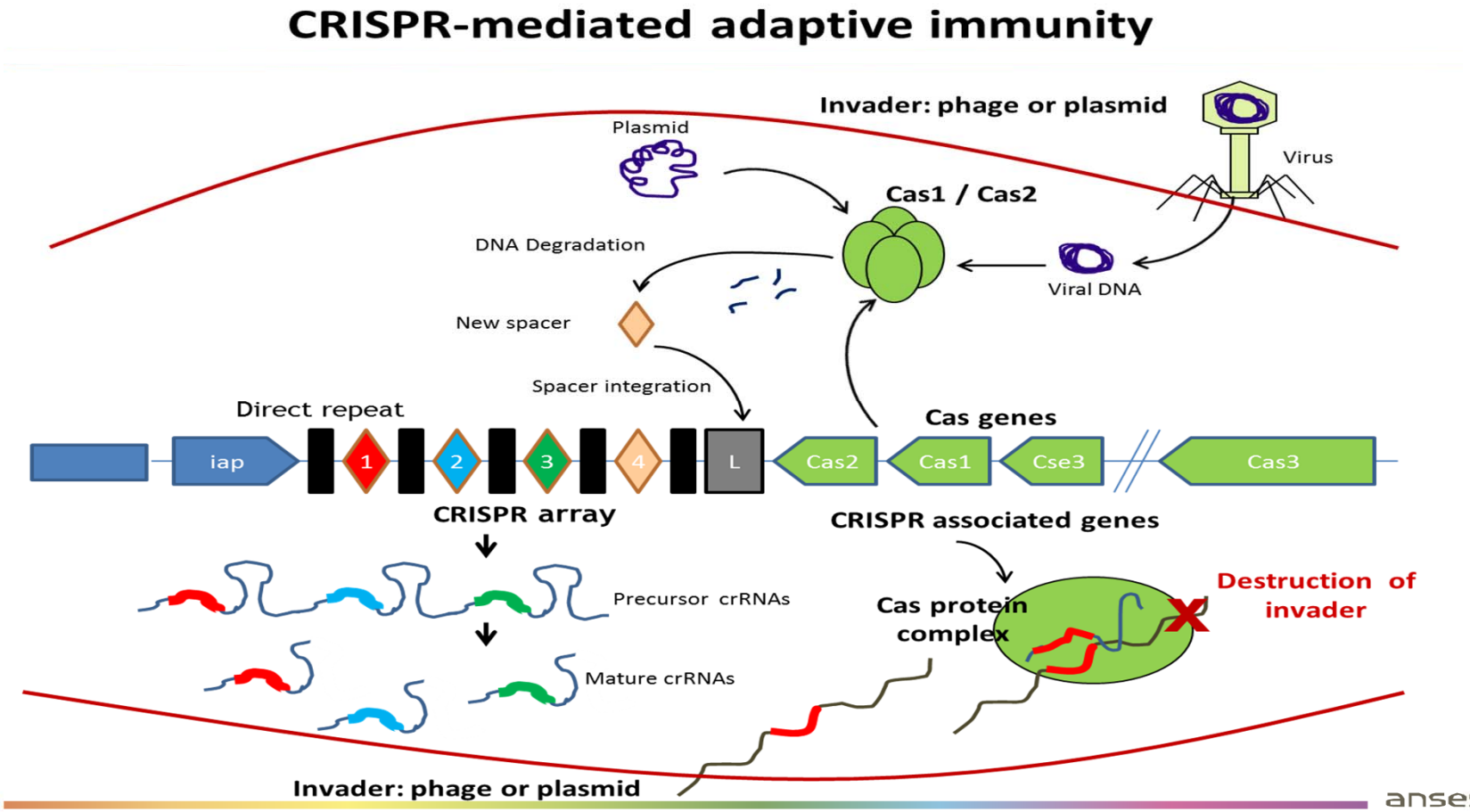
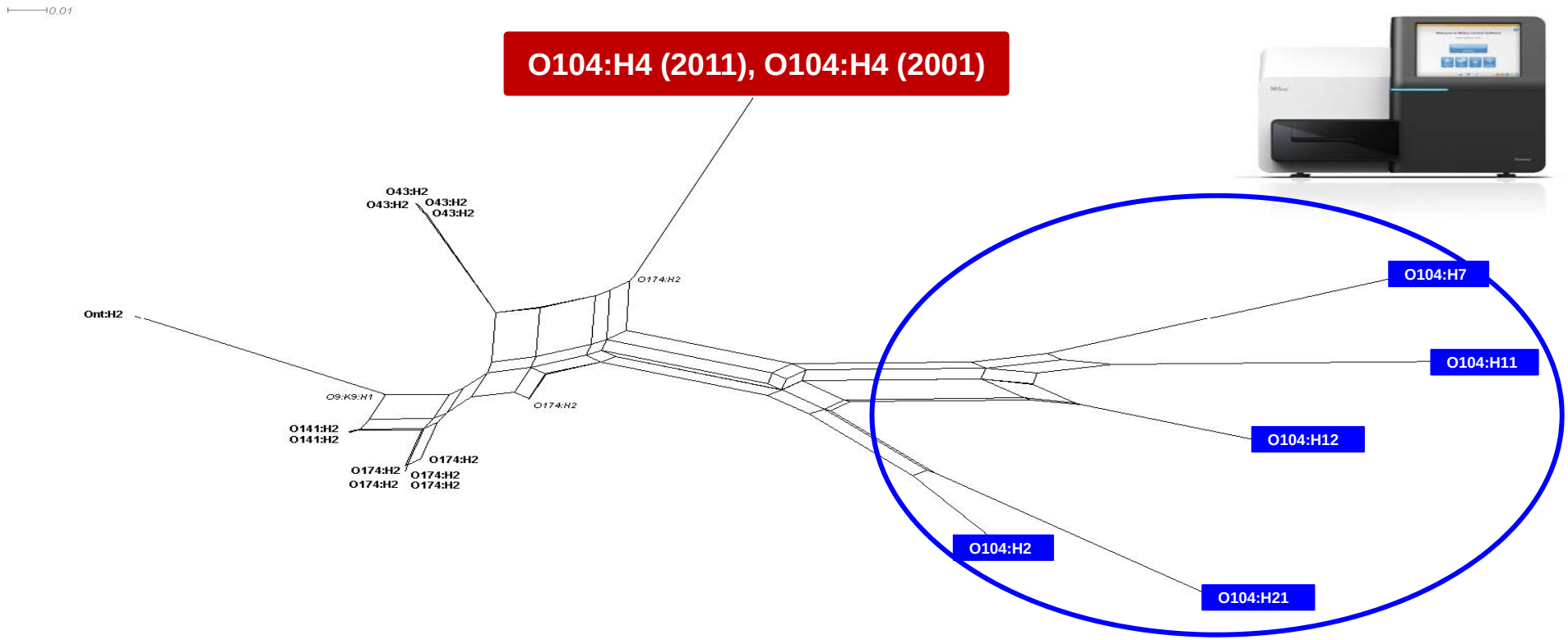
Genotypes and virulence characteristic of O104:H4 from different origins and sources

- The EAEC genetic markers were detected only in the outbreak strains.
- The 2011 outbreak strains and older O104:H4 strains from Germany; Georgia and France have more than 86% similarities and could be categorized in the same cluster.
- O104:H4 strains isolated between 2001 and 2009 are quite different from the 2011 outbreak strains with regard to their plasmid composition.
- Strains of serotypes O104:H7 or O104:H21 isolated in the US or in Europe are really divergent for their stx subtypes, virulence genes and PFGE profile indicating that they have evolved separately from O104:H4.

CRISPR sequencing of *E. coli* O104:H4



Phylogenetic relationship of CRISPR loci



Comparison of the CRISPR loci of O104 strains and other strains having similar CRISPR loci.

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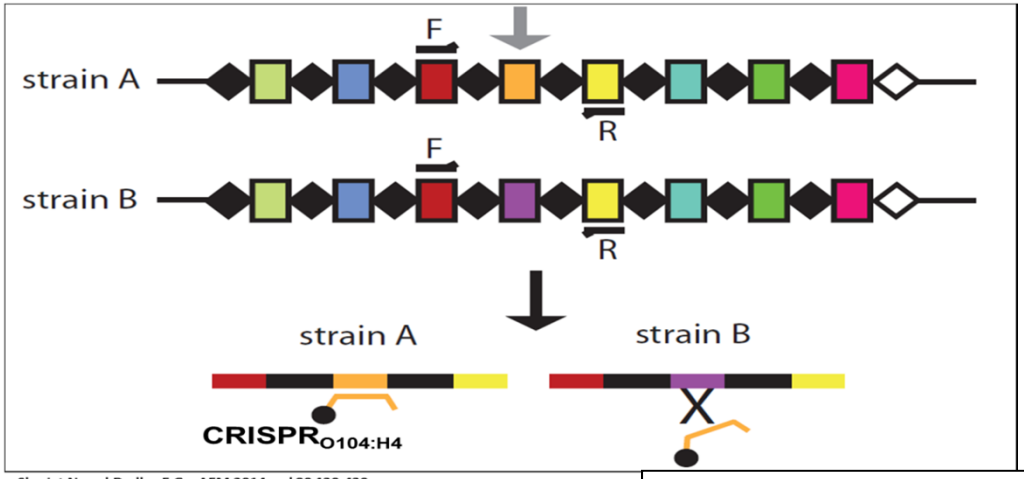
Delannoy *et al*. J. Clin. Microbiol. 2012, 50 (11): 3485-3492

CRISPR loci as specific genetic marker of EHEC O104:H4



Shariat N. and Dudley E.G., AEM 2014, vol 80 430-439

Serotype	Number	PCR assays *aggR*	*wzx*$_{O104}$	*fliC*$_{H4}$	CRISPR$_{O104:H4}$	Pathotype
O104:H4	46	+	+	+	+	EAHEC
O104:H4	1	+	+	+	+	EAEC
Or:H4	1	+	+	+	+	EAEC
O104:H2	3	-	+	-	-	STEC
O104:H2	1	-	+	-	-	EC
O104:H7	5	-	+	-	-	STEC
O104:H7	1	-	+	-	-	EC
O104:H11	2	-	+	-	-	EC
O104:H12	2	-	+	-	-	EC
O104:H21	11	-	+	-	-	STEC / atypical EHEC
O104:H21	1	-	+	-	-	EC
Or:H21	2	-	+	-	-	STEC / atypical EHEC
O8:K9:H10	1	-	+	-	-	EC
O9:K9:H51	1	-	+	-	-	EC
O9:K9:H12	1	-	+	-	-	EC
O9:K9:H1	1	-	+	-	-	EC

100% of EHEC O104:H4 detected

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Specific Detection of Enteroaggregative Hemorrhagic *Escherichia coli* O104:H4 Strains by Use of the CRISPR Locus as a Target for a Diagnostic Real-Time PCR

BfR Anses

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In 2011, a large outbreak of an unusual bacterial strain occurred in Europe. This strain was characterized as a hybrid of an enteroaggregative *Escherichia coli* (EAEC) and a Shiga toxin-producing *E. coli* (STEC) strain of the serotype O104:H4. Here, we present a single PCR targeting the *c*lustered *r*egularly *i*nterspaced *s*hort *p*alindromic *r*epeats locus of *E. coli* O104:H4 ($CRISPR_{O104:H4}$) for specific detection of EAEC STEC O104:H4 strains from different geographical locations and time periods. The specificity of the $CRISPR_{O104:H4}$ PCR was investigated using 1,321 *E. coli* strains, including reference strains for *E. coli* O serogroups O1 to O186 and flagellar (H) types H1 to H56. The assay was compared for specificity using PCR assays targeting different O104 antigen-encoding genes ($wbwC_{O104}$, wzx_{O104}, and wzy_{O104}). The PCR assays reacted with all types of *E. coli* O104 strains (O104:H2, O104:H4, O104:H7, and O104:H21) and with *E. coli* O8 and O9 strains carrying the K9 capsular antigen and were therefore not specific for detection of the EAEC STEC O104:H4 type. A single PCR developed for the $CRISPR_{O104:H4}$ target was sufficient for specific identification and detection of the 48 tested EAEC STEC O104:H4 strains. The 35 *E. coli* O104 strains expressing H types other than H4 as well as 8 *E. coli* strains carrying a K9 capsular antigen tested all negative for the $CRISPR_{O104:H4}$ locus. Only 12 (0.94%) of the 1,273 non-O104:H4 *E. coli* strains (serotypes Ont:H2, O43:H2, O141:H2, and O174:H2) reacted positive in the $CRISPR_{O104:H4}$ PCR (99.06% specificity).

More than 400 serotypes of Shiga toxin (Stx)-producing *Escherichia coli* (STEC) strains have been described as agents of disease in humans, and some of these have been shown to be associated with severe diseases, such as hemorrhagic colitis (HC) and hemolytic-uremic syndrome (HUS). These strains were called enterohemorrhagic *E. coli* (EHEC) and were found to carry additional virulence markers besides Stx, such as effectors encoded by the locus of enterocyte effacement (LEE) and various non-LEE-encoded effectors. A concept of molecular risk assessment (MRA) was developed by Karmali et al. (13) and Coombes et al. (9) that employs PCR for identification of human-pathogenic EHEC. Using the MRA approach for screening STEC collections (6, 8), an increasing number of emerging EHEC types was detected.

During spring 2011, Europe faced its largest STEC outbreak involving an emerging enterohemorrhagic *Escherichia coli* O104:H4 strain (1). This EHEC strain presents an unusual virulence pattern that combines the production of Stx2a with enteroaggregative adherence which is encoded by genes of the pAA plasmid and chromosomally carried genes of enteroaggregative *E. coli* (EAEC) strains (1, 10). This new type of EHEC was designated enteroaggregative hemorrhagic *E. coli* since it shares virulence markers of both EHEC and EAEC strains. On the genome level (5, 17), the strain was found to be most closely related to an EAEC O104:H4 strain, strain 55989, that was isolated in Central Africa in 1995 (11). This hybrid EAEC STEC O104:H4 strain was found to be negative for the LEE-encoded effector and non-LEE-encoded effector (*nle*), both of which are presently being used by the current MRA approach to define human virulent EHEC types. Therefore, new diagnostic approaches needed to be developed for detection of EAEC STEC O104:H4 strains. The lack of unique biochemical traits of the hybrid EAEC STEC O104:H4 strains makes their detection with cultural and phenotypical tests difficult and time-consuming. Therefore, rapid molecular testing methods allowing for timely detection of these strains are deemed highly desirable.

During the course of the O104:H4 outbreak investigation, multitarget PCR assays have been used for rapid screening of samples; however, all of these assays require cultural isolation of the bacteria to confirm that all gene targets are present in the same strain. The used PCR assays (4, 12, 21, 26) combine multiple pairs of primers targeting, for example, genes encoding Shiga toxin 2 (stx_2), O104 (rfb_{O104}) and H4 ($fliC_{H4}$) antigens, tellurite resistance (*terD*), and AggR (*aggR*), which is the master regulator of EAEC plasmid, as well as chromosomally inherited virulence genes (18). However, none of these gene targets was unique to the O104:H4 outbreak strain. Therefore, samples containing a mixed flora of bacteria, such as those collected from environmental and food sources, did not allow prediction that all targets were present in the same bacterial strain. Hence, these assays were suitable only for bacterial isolates and have limited use with clinical, food, or environmental samples.

Based on nucleotide sequence analysis of the genome of EAEC STEC O104:H4, we identified in the *c*lustered *r*egularly *i*nterspaced *s*hort *p*alindromic *r*epeats (CRISPR) locus of the epidemic

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CRISPR loci as specific genetic marker of EHEC O104:H4

- Use of CRISPR loci as specific genetic markers for O104:H4 EHEC strains.
- CRISPR real-time PCRs
  - sensitivity estimates: 100%
  - specificity estimates: 99.06%
  - LOD < 6 cfu.reaction^{-1}
- Strains belonging to the same serogroup but with different H-types tested negative.
- Potential candidate for detection of O104:H4 EHECs in complex matrices such as food samples
  → Evaluation on spiked and naturally contamined samples.

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What BfR and Anses learned from the O104 outbreak :

- A **'task force'** with the **BfR** (NRL for E. coli) and **Anses** (IdentyPath Genomic platform) can be set up to rapidly face the outbreak.
- **BfR** and **Anses** can join their efforts to rapidly design a real-time PCR test specific for the outbreak strain in case of crisis.
- The method developed can be provided to the other European Member States in only few days and a specific test can be commercially available in less than 2 weeks (Pall GeneDisc)
  ---> Routine testing and large scale analysis.
- Based on the molecular detection and typing methods (CRISPR, PFGE) newly developed we determined the **Genotypes and virulence characteristic of O104:H4 from different origins and sources.**
- Importance to anticipate the crisis by developing research projects, by exchange of expertise & material and by sharing PhD student

What next at the BfR and Anses after the O104 outbreak ?

Many common scientific papers were published by the BfR and Anses after this story :

1- Molecular characterization of other *E. coli* serotypes

Feng PCH, **Delannoy S**, Lacher DW, Bosilevac JM, **Fach P**, **Beutin L**. Characterization of Shiga toxin-producing Escherichia coli strains of O91 serogroup isolated from food and environmental samples. Appl Environ Microbiol. 2017 Jul 7. pii: AEM.01231-17.

Miko A, Rivas M, Bentancor A, **Delannoy S**, **Fach P**, **Beutin L**. Emerging types of Shiga toxin-producing E. coli (STEC) O178 present in cattle, deer, and humans from Argentina and Germany. Front Cell Infect Microbiol. 2014 Jun 17;4:78.

Feng PC, **Delannoy S**, Lacher DW, Dos Santos LF, **Beutin L**, **Fach P**, Rivas M, Hartland EL, Paton AW, Guth BE. Genetic diversity and virulence potential of shiga toxin-producing Escherichia coli O113:H21 strains isolated from clinical, environmental, and food sources. Appl Environ Microbiol. 2014 Aug;80(15):4757-63.

Piazza RM, **Delannoy S**, **Fach P**, Saridakis HO, Pedroso MZ, Rocha LB, Gomes TA, Vieira MA, **Beutin L**, Guth BE. Molecular and phenotypic characterization of Escherichia coli O26:H8 among diarrheagenic E. coli O26 strains isolated in Brazil. Appl Environ Microbiol. 2013 Nov;79(22):6847-54.

What next at the BfR and Anses after the O104 outbreak ?

2- Sequencing of the CRISPR array of E. coli

Delannoy S, Beutin L, Fach P. Improved traceability of Shiga-toxin-producing Escherichia coli using CRISPRs for detection and typing. Environ Sci Pollut Res Int. 2016 May;23(9):8163-74.

Delannoy S, Beutin L, Fach P. Use of clustered regularly interspaced short palindromic repeat sequence polymorphisms for specific detection of enterohemorrhagic Escherichia coli strains of serotypes O26:H11, O45:H2, O103:H2, O111:H8, O121:H19, O145:H28, and O157:H7 by real-time PCR. J Clin Microbiol. 2012 Dec;50(12):4035-40.

Delannoy S, Beutin L, Burgos Y, Fach P. Specific detection of enteroaggregative hemorrhagic Escherichia coli O104:H4 strains by use of the CRISPR locus as a target for a diagnostic real-time PCR. J Clin Microbiol. 2012 Nov;50(11):3485-92.

What next at the BfR and Anses after the O104 outbreak ?

3- Molecular serotyping of *E. coli* : Sequencing the O and H antigen genes

Delannoy S, Beutin L, Mariani-Kurkdjian P, Fleiss A, Bonacorsi S, Fach P. The Escherichia coli Serogroup O1 and O2 Lipopolysaccharides Are Encoded by Multiple O-antigen Gene Clusters. Front Cell Infect Microbiol. 2017 Feb 7;7:30. doi:10.3389/fcimb.2017.00030. eCollection 2017.

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What next at the BfR and Anses after the O104 outbreak ?

4- Molecular Risk Assessment (MRA)

Delannoy S, Beutin L, Fach P. Discrimination of enterohemorrhagic Escherichia coli (EHEC) from non-EHEC strains based on detection of various combinations of type III effector genes. J Clin Microbiol. 2013 Oct;51(10):3257-62.

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What next at the BfR and Anses after the O104 outbreak ?

5- A new detection approach for detecting EHEC in food samples

Delannoy S, Chaves BD, Ison SA, Webb HE, Beutin L, Delaval J, Billet I, Fach P. Revisiting the STEC Testing Approach: Using espK and espV to Make Enterohemorrhagic Escherichia coli (EHEC) Detection More Reliable in Beef. Front Microbiol. 2016 Jan 22;7:1.

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   - Japan: patent n° 6166365, 30 June 2017
   - International PCT PCT/IB2013/054888 (WO2013 186754) 14 June 2013.
   - European Patent EP 12171941.3 14 June 2012.

2. **FACH P., BUGAREL M., BEUTIN L.**
   Assay for determining a molecular risk assessment of a complex polymicrobial sample suspected to contain an EHEC.
   Patents published in 2016 in the following countries:
   - Australia: 30 June 2016, n°2010283452
   - Mexico: 01 August 2016, n° 340945
   - United States: 16 August 2016, n°9 416 425
   - Japan: 19 August 2016, n° 5990099
   - Europe: 30 November 2016, n° 2464746
   - Brazil: 22 November 2016, n° 2394

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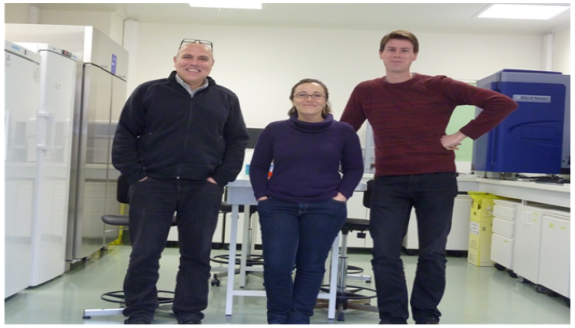

Staff of the IdentyPath genomic platform in Anses

PhD student shared by Anses and BfR (2010-2012)

Staff of the National Reference Laboratory for *E. coli* at the BfR