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In their article Bielaszewska *et al.*[1] have characterized the virulence profile of Shigatoxin-producing *Escherichia coli* (STEC) O104:H4 isolates from 80 patients in the large outbreak in Germany. Here we present microbiological data of the outbreak of bloody diarrhea (BD) and haemolytic and uraemic syndrome (HUS) associated with consumption of sprouts that occurred in June-July 2011 in the Bordeaux area, south-west France. From June 8, 2011 to July 11, 2011, a total of 12 confirmed cases of Shiga-toxin-producing *E. coli* (STEC) infections (HUS, n=9; BD, n=1, simple diarrhoea, n=2) were reported in relation to this outbreak.

The strains were isolated by using Drigalski agar (Bio-Rad, Marne la Coquette, France), cefixime-tellurite sorbitol Mac Conkey agar (CT-SMAC, bioMérieux, La Balme les Grottes, France), CHROMagar STEC (CHROMagar, Paris, France) and extended-spectrum beta-lactamase (ESBL) agar plates (ChromID ESBL, bioMérieux; CHROMagar STEC-O104, CHROMagar) after stool enrichment with GN (Gram-Negative) broth.

PCR screening for STEC virulence factors genes was positive for the *stx*2 gene (*stx2*a variant), whereas *stx*1, *eae*, and EHEC-*hlyA* genes were negative. [2] PCR for enteroaggregative *E. coli* (EAEC) virulence factors was positive for *agg*R and *pic*, whereas *ast*A was negative. [3]

Phylogenetic grouping determinated by a PCR-based method showed that the strains belonged to the group B1. [4] Serotyping revealed that the strains belonged to the O104:H4 serotype. PCR screening for extra-intestinal virulence genes was positive for *irp*2, *fuy*A, and *aerobactin* genes. [4]

All strains were resistant to ampicillin, third generation cephalosporins (ESBL production), streptomycin, nalidixic acid, tetracycline and cotrimoxazole but susceptible to carbapenems, ciprofloxacin, chloramphenicol, kanamycin and gentamicin. PCR analysis and sequencing revealed that the ESBL phenotype was due to the $bla_{\text{CTX-M-15}}$ gene.

Genomic comparison of the French and German outbreak strains was performed by using pulsed-field gel electrophoresis (*Xba*I), semi-automated rep-PCR (Diversilab, bioMérieux), and by optical mapping (kindly provided by OpGen, Gaithersburg, MD, USA, with a support of Phylogene, Nîmes, France). [2,4,5] The three methods showed that the strains from the French and the German 2011 outbreaks were genetically related, whereas two Shiga-toxin-producing O104:H4 strains isolated earlier (2004 and 2009) in France were not genetically related to the 2011 strains (Figure).

In conclusion, the French and German outbreak strains were identical. They combine the Shiga-toxin 2 gene, an unusual EAEC genetic background, and the ESBL $bla_{\text{CTX-M-15}}$ gene. The association of this very rare strain with sprouts in France and Germany within a short period evokes a common origin, likely contaminated seeds of a single type.

We declare that we have no conflict of interest.

References

1. Bielaszewska M, Mellmann A, Zhang W, Köck R, Fruth A, Bauwens A, Peters G, Karch H. Characterisation of the *Escherichia coli* strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. *Lancet Infect Dis* 2011, epub ahead of print.

2. Bidet Ph, Mariani-Kurkdjian P, Grimont F, Brahimi N, Courroux C, Grimont P, Bingen E. Characterization of Escherichia coli O157:H7 isolates causing haemolytic uraemic syndrome in France. *J Med Microbiol* 2005; **54**: 71-5.

3. Müller D, Greune L, Heusipp G, Karch H, Fruth A, Tshäpe H, Schmidt MA. Identification of unconventional intestinal pathogenic *Escherichia coli* isolates expressing intermediate virulence factor profiles by using a novel single-step multiplex PCR. *Appl Environ Microbiol* 2007; **73**: 3380-90.

4. Bonacorsi S, Bidet Ph, Mahjoub F, Mariani-Kurkdjian P, Ait-Ifrane S, Courroux C, Bingen E. Semi-automated rep-PCR for rapid differentiation of major clonal groups of *Escherichia coli* meningitis strains. *Int J Med Microbiol* 2009; **299**: 402-9.

5. Mellmann A, Harmsen D, Cummings CA, Zentz EB, Leopold SR, Rico A, Prior K, Szczepanowski R, Ji Y, Zhang W, McLaughlin SF, Henkhaus JK, Leopold B, Bielaszewska M, Prager R, Brzoska PM, Moore RL, Guenther S, Rothberg JM, Karch H. Prospective Genomic Characterization of the German Enterohemorrhagic *Escherichia coli* O104:H4 Outbreak by Rapid Next Generation Sequencing Technology. *PLoS One* 2011;**6**:e22751.

Figure Legend

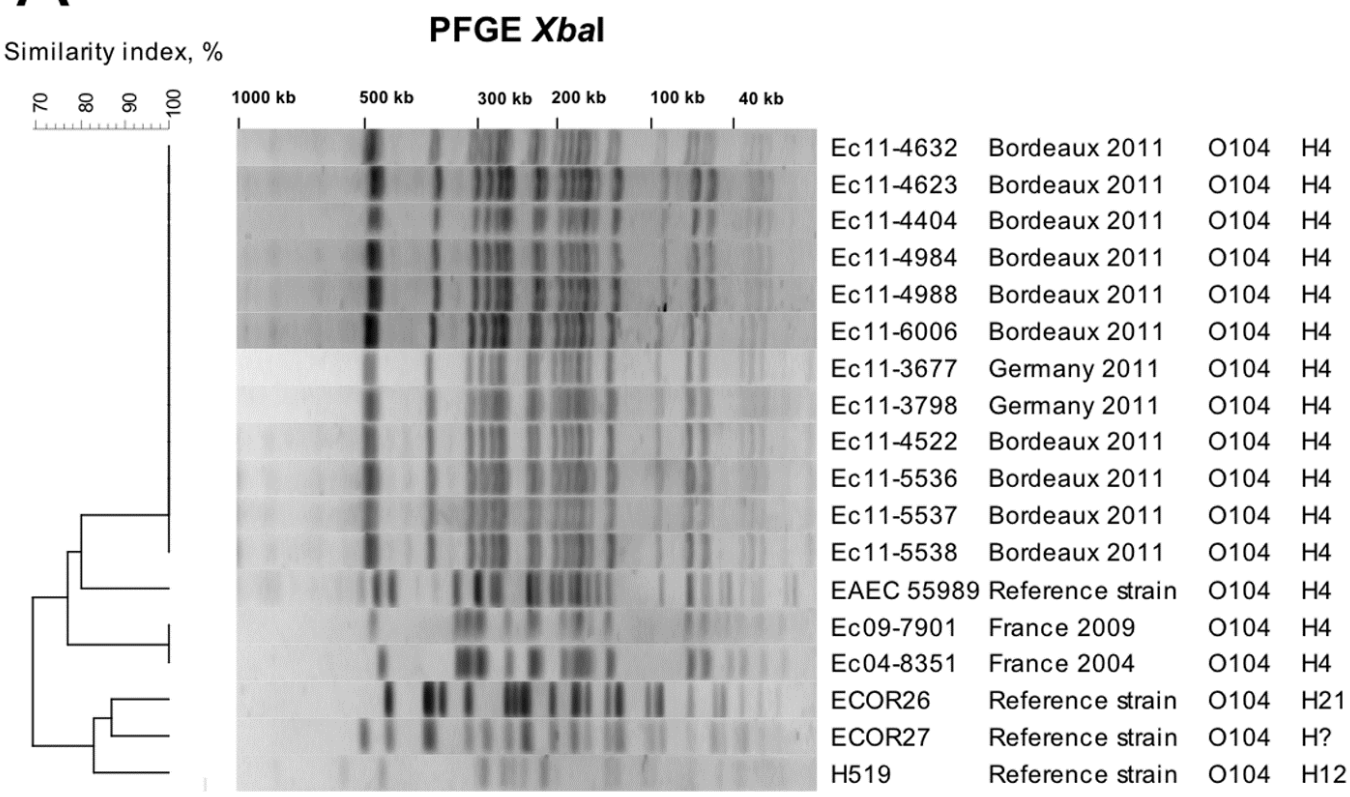
Panel A. *Xba*I-pulsed field gel electrophoresis (PFGE) profiles obtained from 9 STEC O104:H4 from Bordeaux, France (2011), two from Germany (2011), two from France (2004 and 2009), and from various *E. coli* O104 reference strains. The dendrograms generated by BioNumerics version 6.5 software (Applied Maths, Sint-Martens-Latem, Belgium) show the

results of cluster analysis on the basis of PFGE fingerprinting. Similarity analysis was performed using the Dice coefficient and clustering was done using the unweighted pair-group method with arithmetic averages (UPGMA).

Panel B. A comparison of an Optical Map from a Bordeaux 2011 outbreak representative strain (Ec11-5598) to an Optical Map from a German 2011 outbreak strain (Ec11-3798) (**Top)**. A comparison of an Optical Map from a Bordeaux 2011 outbreak representative strain (Ec11-5598) to an Optical Map from an epidemiologically-unrelated STEC O104:H4 isolated in 2009 **(Bottom)**. *De novo* whole genome Optical Maps were created using the Argus® Optical Mapping System with the *Nco*I restriction enzyme. Maps were imported into MapSolver™ software and compared to one another. Restriction enzyme cut sites are represented by vertical black bars, and the spaces between the bars represent restriction fragments. Blue shaded fragments are shared between the Optical Maps indicating whole genome alignment which supports the clonality of these isolates. White regions of the Maps are unique to the isolate containing those regions.

Panel C. A comparison of Optical Maps of selected *E. coli* O104:H4 strains. Optical Maps were compared and then clustered (MapSolver™) using UPGMA based on the resulting pairwise distance metrics. Scale represents percent map difference.

A


PFGE XbaI
Similarity index, %
70
80
90
100
1000 kb
500 kb
300 kb
200 kb
100 kb
40 kb
Ec11-4632 Bordeaux 2011 O104 H4
Ec11-4623 Bordeaux 2011 O104 H4
Ec11-4404 Bordeaux 2011 O104 H4
Ec11-4984 Bordeaux 2011 O104 H4
Ec11-4988 Bordeaux 2011 O104 H4
Ec11-6006 Bordeaux 2011 O104 H4
Ec11-3677 Germany 2011 O104 H4
Ec11-3798 Germany 2011 O104 H4
Ec11-4522 Bordeaux 2011 O104 H4
Ec11-5536 Bordeaux 2011 O104 H4
Ec11-5537 Bordeaux 2011 O104 H4
Ec11-5538 Bordeaux 2011 O104 H4
EAEC 55989 Reference strain O104 H4
Ec09-7901 France 2009 O104 H4
Ec04-8351 France 2004 O104 H4
ECOR26 Reference strain O104 H21
ECOR27 Reference strain O104 H?
H519 Reference strain O104 H12