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## **Escherichia coli O104:H4 south-west France, June 2011**

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2 *Escherichia coli* O104:H4 south-west France, June 2011  
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25 In their article Bielaszewska *et al.*<sup>1</sup> have characterized the virulence profile of  
26 Shiga-toxin-producing *Escherichia coli* (STEC) O104:H4 isolates from 80 patients in the large  
27 outbreak in Germany. Here we present microbiological data of the outbreak of bloody  
28 diarrhea (BD) and haemolytic and uraemic syndrome (HUS) associated with consumption of  
29 sprouts that occurred in June-July 2011 in the Bordeaux area, south-west France. From June  
30 8, 2011 to July 11, 2011, a total of 12 confirmed cases of Shiga-toxin-producing *E. coli*  
31 (STEC) infections (HUS, n=9; BD, n=1, simple diarrhoea, n=2) were reported in relation to  
32 this outbreak.

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

38 PCR screening for STEC virulence factors genes was positive for the *stx2* gene (*stx2a*  
39 variant), whereas *stx1*, *eae*, and EHEC-*hlyA* genes were negative.<sup>2</sup> PCR for enteroaggregative  
40 *E. coli* (EAEC) virulence factors was positive for *aggR* and *pic*, whereas *astA* was negative.<sup>3</sup>

41 Phylogenetic grouping determined by a PCR-based method showed that the strains  
42 belonged to the group B1.<sup>4</sup> Serotyping revealed that the strains belonged to the O104:H4  
43 serotype. PCR screening for extra-intestinal virulence genes was positive for *irp2*, *fuyA*, and  
44 *aerobactin* genes.<sup>4</sup>

45 All strains were resistant to ampicillin, third generation cephalosporins (ESBL  
46 production), streptomycin, nalidixic acid, tetracycline and cotrimoxazole but susceptible to  
47 carbapenems, ciprofloxacin, chloramphenicol, kanamycin and gentamicin. PCR analysis and  
48 sequencing revealed that the ESBL phenotype was due to the *bla*<sub>CTX-M-15</sub> gene.

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

56  
57 In conclusion, the French and German outbreak strains were identical. They combine the  
58 Shiga-toxin 2 gene, an unusual EAEC genetic background, and the ESBL *bla*<sub>CTX-M-15</sub> gene.  
59 The association of this very rare strain with sprouts in France and Germany within a short  
60 period evokes a common origin, likely contaminated seeds of a single type.

61  
62 We declare that we have no conflict of interest.

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## 90 91 92 93 **Figure Legend**

94  
95 **Panel A.** *Xba*I-pulsed field gel electrophoresis (PFGE) profiles obtained from 9 STEC  
96 O104:H4 from Bordeaux, France (2011), two from Germany (2011), two from France (2004  
97 and 2009), and from various *E. coli* O104 reference strains. The dendrograms generated by  
98 BioNumerics version 6.5 software (Applied Maths, Sint-Martens-Latem, Belgium) show the

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

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

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

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