Presence of Enterohemorrhagic Escherichia coli ST678/O104:H4 in France prior to 2011

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Abstract

Two isolates of Enterohemorrhagic *Escherichia coli* O104:H4 were isolated in France in 2004 and 2009. Both were characterized and compared to the strain which caused the German outbreak in 2011 and to other O104:H4 strains. This suggests a presence of different O104:H4 EHEC several years prior to the 2011 outbreak.
From May to July 2011, a large scale outbreak of Enterohemorrhagic *Escherichia coli* (EHEC) has been observed in several European countries, but mainly affecting Northern Germany. Altogether, about 4000 cases of EHEC infections and nearly 50 fatalities have been reported (http://www.promedmail.org/pls/apex/f?p=2400:1001:2252791428282352::NO::F2400_P1001_BACK_PAGE,F2400_P1001_PUB_MAIL_ID:1010,89282). This outbreak also led to massive economic damage for farmers and to the irritation of the public, after various vegetables and sprouts as well as a possible bioterrorist attack were publicly discussed as possible sources of the infection. Recent epidemiological studies singled out imported fenugreek seeds although this was not substantiated yet by laboratory evidence (http://www.rki.de/cln_117/nn_467482/DE/Content/Service/Presse/Pressemitteilungen/2011/09_2011.html).

In order to understand the evolution, and possibly, the provenance of the recent outbreak strain, it is of high interest to identify related or ancestral isolates. Only few cases of infections caused by *E. coli* O104:H4 have yet been described earlier than 2011. One was a case of Hemolytic-Uremic Syndrome (HUS) from South Korea (1), but the causative strain appeared not to be closely related to the current European outbreak strain differing in toxin carriage, resistance properties and pulsed field gel electrophoresis (8). Another one originated from Italy, 2009 (16). A recently described and fully sequenced strain HUSEC 41, isolate 01-09591, originated from a HUS case in Germany in 2001 (4, 9).

Prior to the 2011 outbreak, *E. coli* O104:H4 was also detected in two isolated clinical cases from France. One was a 6 years old male child with HUS from the Lyon area in 2009. He was hospitalized, treated with azithromycin, and cured. The other one was an adult male patient with hemorrhagic colitis from the town of Lille, in Northern France, in 2004. The clinical
course and outcome are not known. Both isolates were characterized by microarray analysis using a previously described system (7), and they yielded identical hybridization patterns. They also were compared to the current European outbreak strain as represented by thirteen identical isolates. These were three serial isolates from a young female patient from Dresden, Saxony, who was admitted with bloody diarrhea and HUS after travelling to Northern Germany, and 10 isolates from clinical cases from North-Eastern Germany (courtesy of D. Bandt, Frankfurt/Oder).

Recently, the similarity of E. coli 55989, an enteroaggregative strain that was isolated in Central Africa in the late 1990s (11, 17), to the European outbreak strain (GenBank entries AFOB00000000, GL989507.1) was noted based on genome sequence data (4, 5) and particularly, identical MLST alleles (ST678, see https://github.com/ehec-outbreak-crowdsourced/BGI-data-analysis/wiki/MLST-and-serotyping-55989-in-silico and (4)). Thus, this strain as well as the German HUSEC 41 isolate 01-09591 (4) were included into the comparison based on analyses of their genome sequences (GenBank CU928145 and AFPS01000000, respectively).

The two French O104:H4 isolates belonged, as E. coli 55989, the German HUSEC 41 isolate 01-09591 and the European outbreak strain, to sequence type 678. All these strains carried wzx-O104 and fliC-H04 determinants as well as genes encoding a major fimbrial subunit (lpfA) and secreted serine proteases (pic, sepA, sigA). Moreover, they were characterized by the absence of eae (intimin, locus of enterocyte effacement) and of Shiga-like toxin 1 (stx- A/B1).

The two French isolates carried Shiga-like toxin 2 (stx-A/B2) genes and the microcin operon (mchB, mchC, mchF). These genes were also present in all tested isolates of the outbreak
strain, in its published genome sequences as well as in the German HUSEC 41 isolate 01-09591. They were absent from the *E. coli* 55989 genome sequence.

All strains carried *aggR* (gene encoding aggregative-adherence fimbriae, for the French isolates determined by PCR (12)) but there were differences with regard to the carriage of pAA plasmids. The two French isolates were positive for *astA* (enteroaggregative heat stable enterotoxin), which was absent from isolates of the European outbreak strain. It can be assumed that the French isolates carry a pAA plasmid similar or identical to those of *E. coli* 55989 (GenBank AF411067.1) and 01-09591 (4) which include *astA* as well as a type I (14, 15) *aggDCBA* operon. In contrast, genome sequences of the outbreak strain indicate the presence of a type III operon (2) and the lack of *astA*.

Further differences included the carriage of genes associated with resistance toward antibiotic compounds. The two French isolates carried none of the resistance genes covered by the array (7). The 2004 isolate was completely susceptible, whereas the 2009 isolate was resistant to nalidixic acid. The European outbreak strain harbored an extended spectrum beta-lactamase gene *bla*<sub>CTX-M-15</sub> (4,6) which is currently spreading worldwide across populations of different enterobacteria (13). Beside, this strain also carried an additional beta-lactamase gene *bla*<sub>TEM-1</sub>, genes encoding dihydrofolate reductase type 7 (*dfrA7*) and dihydropteroate synthetase type 1+2 (*sul1*, *sul2*), streptomycin resistance genes *strA* (*apha3*) and *strB* (*apha6*) as well as a gene for a tetracycline efflux protein (*tetA*). Analyses of the genome sequences showed that *E. coli* 55989 harbored the tetracycline resistance gene *tetB* while 01-09591 was positive for *strA*, *strB*, *sul2* and *bla*<sub>TEM-1</sub>.

In conclusion, the two French isolates described herein are largely identical to the recently described HUSEC 41 isolate 01-09591 from Germany. It is tempting to regard these three isolates as some kind of intermediate form or missing link between the European outbreak
strain and its putative ancestor, an *E. coli* 55989-like EAEC strain. However, there are differences in *astA/aggR* plasmids, with the 2011 outbreak strain carrying another pAA than all other O104:H4, as well as in pulsed field gel electrophoresis profiles (Figure). It is also noteworthy that the French isolates lack *bla*\_TEM-1 and the other resistance genes detected in 01-09591 although they have been isolated more recently. Therefore, it is unlikely that a direct line of ancestry lead from *E. coli* 55989 through 01-09591 and the French isolates to the recent outbreak strain. As proposed previously (10), it can be assumed that several O104:H4 EHEC lineages emerged from O104:H4 EAEC ancestors which differ most obviously in pAA and resistance gene carriage. Sporadic observations from France, Germany (4, 9), Italy (16) and Korea (8) indicate that several such strains might have persisted for several years without raising much attention, apparently due to their rarity. Contrarily to other EHEC, there are no reports on a presence of O104:H4 EHEC in cattle. This does not rule out a very rare occurrence, a presence in other animals, or in cows from other parts of the world. However, because of the lack of such reports and because of their EAEC parentage (5) it should be investigated whether humans rather than cattle were the actual reservoir of O104:H4.
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References


Figure 1: 

Not I (3) and Xba I pulsed-field gel electrophoresis patterns of the 2011 European outbreak strain, the isolate from Lyon, 2009, and the isolate from Lille, 2004.
M: PulseNet marker

**NotI PFGE profiles**
1: O104:H4 outbreak strain, 2011
2: O104:H4 isolate from Lyon, 2009
3: O104:H4 isolate from Lille, 2004

**XbaI PFGE profiles**
4: O104:H4 outbreak strain, 2011
5: O104:H4 isolate from Lyon, 2009
6: O104:H4 isolate from Lille, 2004