

Presence of Enterohemorrhagic *Escherichia coli* ST678/O104:H4 in France Prior to 2011[∇]

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Two isolates of enterohemorrhagic *Escherichia coli* (EHEC) 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 that different O104:H4 EHEC strains were present several years prior to the 2011 outbreak.

From May to July 2011, a large-scale outbreak of enterohemorrhagic *Escherichia coli* (EHEC) was observed in several European countries, mainly affecting northern Germany. Altogether, about 4,000 cases of EHEC infections and nearly 50 fatalities were reported (8). This outbreak also led to massive economic losses for farmers and to widespread public concern, 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 has not yet been substantiated by laboratory evidence (6).

In order to understand the evolution and possibly the origin of the recent outbreak strain, it is of great interest to identify related or ancestral isolates. Only a few cases of infections caused by *E. coli* O104:H4 were 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 profiles (10). Another one originated from Italy in 2009 (18). A recently described and fully sequenced strain, HUSEC 41 isolate 01-0991, originated from a HUS case in Germany in 2001 (4, 11).

Prior to the 2011 outbreak, *E. coli* O104:H4 was also detected in two isolated clinical cases in France. One patient was a 6-year-old male child from the Lyon area treated for HUS 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 (9), and they yielded identical hybrid-

ization patterns. They were also compared to the current European outbreak strain, as represented by 13 identical isolates. These were three serial isolates from a young female patient from Dresden, Saxony, Germany, who was admitted with bloody diarrhea and HUS after traveling to northern Germany, and 10 isolates from clinical cases from northeastern Germany (provided by D. Bandt, Frankfurt/Oder, Germany).

Recently, the similarity of *E. coli* 55989, an enteroaggregative strain that was isolated in Central Africa in the late 1990s (13, 19), to the European outbreak strain (GenBank entries AFOB00000000 and GL989507.1) was noted based on genome sequence data (4, 5) and particularly identical multilocus sequence typing alleles (ST678) (<https://github.com/ehec-outbreak-crowdsourced/BGI-data-analysis/wiki/MLST-and-serotyping-55989-in-silico> and reference 4). Thus, this strain and the German HUSEC 41 isolate 01-0991 (4) were included in the comparison based on analyses of their genome sequences (GenBank numbers CU928145 and AFPS01000000, respectively).

Like *E. coli* 55989, the German HUSEC 41 isolate 01-0991, and the European outbreak strain, the two French O104:H4 isolates belonged 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*, and *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*, and *mchF*). These genes were also present in all tested isolates of the outbreak strain and in its published genome sequences, as well as in the German HUSEC 41 isolate 01-0991. They were absent from the *E. coli* 55989 genome sequence.

All strains carried *aggR* (encoding aggregative-adherence fimbriae; determined for the French isolates by PCR [14]), 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 as-

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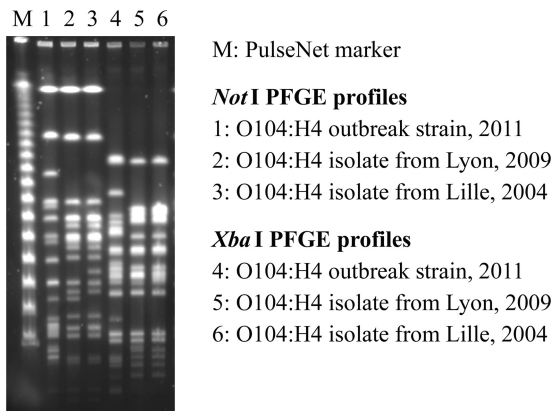


FIG. 1. NotI (3) and XbaI pulsed-field gel electrophoresis patterns of the 2011 European outbreak strain, the isolate from Lyon (2009), and the isolate from Lille (2004).

sumed that the French isolates carry a pAA plasmid similar or identical to those of *E. coli* 55989 (GenBank AF411067.1) and 01-0991 (4), which include *astA* as well as a type III (2) *aggDCBA* operon. In contrast, genome sequences of the outbreak strain indicate the presence of a type I operon (16, 17) 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 (9). The 2004 isolate was completely susceptible to nalidixic acid, whereas the 2009 isolate was resistant. The European outbreak strain harbored an extended-spectrum- β -lactamase gene, *bla*_{CTX-M-15} (4, 7), which is currently spreading worldwide across populations of different enterobacteria (15). This strain also carried an additional β -lactamase gene (*bla*_{TEM-1}), genes encoding dihydrofolate reductase type 7 (*dfrA7*) and dihydropteroate synthetase types 1 and 2 (*sul1* and *sul2*), and 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 01-0991 was positive for *strA*, *strB*, *sul2*, and *bla*_{TEM-1}.

In conclusion, the two French isolates described herein are largely identical to the recently described HUSEC 41 isolate 01-0991 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*- and *aggR*-bearing plasmids, with the 2011 outbreak strain carrying another pAA than all other O104:H4 isolates, as well as in pulsed-field gel electrophoresis profiles (Fig. 1). It is also noteworthy that the French isolates lack *bla*_{TEM-1} and the other resistance genes detected in 01-0991, although they were isolated more recently. Therefore, it is unlikely that a direct line of ancestry leads from *E. coli* 55989 through 01-0991 and the French isolates to the recent outbreak strain. As proposed previously (12), 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, 11), Italy (18), and Korea (10) indicate that sev-

eral such strains might have persisted for several years without receiving much attention, apparently due to their rarity. In contrast to other EHEC strains, there are no reports of an O104:H4 EHEC strain isolated from cattle. This does not rule out a very rare occurrence, a presence in other animals, or a presence 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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