

E. coli 0104:H4 - The Outbreak Strain in Germany

From May to June 2011 there was an outbreak of 3,602 cases of diarrhea caused by Shiga-toxin-producing *Escherichia coli* (STEC). The outbreak originated from northern Germany and resulted in 908 cases of hemolytic uremic syndrome (HUS) and 47 deaths (1,2). The source of the outbreak was contaminated raw fenugreek sprouts. According to the Robert Koch Institut the peak of the outbreak was May 22, 2011. The outbreak strain affected primarily women, resulting in severe neurological complications (3,4). Properties of 0104:H4 outbreak strain include:

- Incubation of 8 days
- HUS developed 5 days after symptoms, compared to 7 days for 0157(1)
- Resistant to ampicillin, cefotaxime, ceftazidime, streptomycin, sulfamethoxazole, trimethoprim, cotrimoxazole, tetracycline, nalidixic acid
- Sensitive to imipenem, kanamycin, gentamicin, chloramphenicol, and ciprofloxacin (4).
- Ferments sorbitol

The Germany outbreak strain was identified as serotype O104:H4 and contained virulence features that were seen in enteroaggregative *E. coli* (EAEC) and enterohemorrhagic *E. coli* (EHEC). EAEC are known to cause persistent diarrhea in infants and children in developing countries (5) and EHEC is well known as a food borne pathogen causing diarrhea after contaminated food is ingested. The current outbreak strain was missed by standard procedures that include Sorbitol MacConkey (SMAC) plates (4).

The pAA plasmid carried by the outbreak strain encodes (i) aggregative adherence fimbriae (AAF/I), (ii) Aat complex (enteroaggregative ABC transporter that transports dispersin onto the bacterial cell surface), (iii) the dispersin protein (to control electrostatic attraction between the AAF and bacterial surface), and (iv) AggR (regulates AAF) that are all characteristics of EAEC (2, 6). The *E. coli* O104:H4 contains a toxin-encoding phage that is similar to 933W phage found in EHEC (2) but with one nucleotide change in each of the subunits (Stx2A and Stx2B) (4). The Germany outbreak strain contains another virulence factor referred to SPATEs (Serine Protease Autotransporter Toxins). The O104:H4 strain has a combination of SPATEs that include SepA,

SigA, and PIC. PIC promotes colonization of the gut by clearing the mucin from epithelial cells. SigA causes rounding of enterocytes by clearing the cytoskeletal protein spectrin which maintains the plasma membrane and cytoskeletal structure of cells. SepA function is not known. EAEC usually do not code for more than two SPATEs unlike the outbreak strain. Strain O104:H4 also had virulence factors that included long polar fimbriae (Ipf) and *iha* homologue adhesion that are associated with EHEC and colonization of the gut. The O104:H4 outbreak strain contained plasmid pESBL that encodes for extended spectrum beta lactase CTX-M-15 that is a recent addition and not in the 2001 strain, but the antibiotic susceptibility remained the same between both strains (2).

A serotype O104:H4 was previously identified in 2001 in Germany (01-09591; HUSECO41) and 2002 in Central Africa (55989). The O104:H4 strain from Germany is referred to as LB226692. The 2011 strain was similar to EAEC strain O104:H4 from Central Africa (55989) (7) and is not the first EAEC to acquire the Stx2 phage (e.g., O111:H2) (2,8).

In 2011, Mellmann proposed two evolutionary models to describe the origin of the 2011 outbreak strain that included (i) the common ancestor model suggesting a O104 progenitor or (ii) the linear ancestry model that suggest that all EHEC O104:H4 originated from the prototypic EAEC 55989. They believe the common ancestor model is probably the most likely situation in this case. The 55989 strain was more than likely derived from a progenitor STEC O104:H4. The 55989 strain has an *stx* integration site at *wrbA* and carries *iha* (9,10). The 55989 strain was formed by six insertion events, whereas LB226692 was formed by three insertion events and 01-09591 was formed by one insertion event. The 2001 isolate kept the AAF/III fimbriae that was also in 55989 and obtained the type IV pilus and TEM-1. The German 2011 O104:H4 strain obtained plasmids containing AAF/I fimbriae, TEM-1 and CTX-M-15 beta lactamase and lost the AAF/III fimbriae (9). Overall, with the additions of CTX-M-15, AAF/I and the combinations of virulence factors from EAEC and EHEC, this O104:H4 strain is highly virulent.

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