Source: Holland, Laue (Robert Koch Institute)





O104:H4 outbreak strain: really a "new bug" ?

6th Annual Workshop of the National Reference Laboratories for E. coli in the EU 4th November, 2011 **FLEMMING SCHEUTZ** WHO Collaborating Centre for Reference and **Research on Escherichia and Klebsiella** Statens Serum Institute (SSI), Denmark





VTEC infection

Haemolytic uraemic syndrome (?%)

Days: -3-2-1012345678InfectionIncubationDiarrheaBloody diarrhea?

vtx+

Clinical course

Spontaneous recovery (~85%)

9

Antibiotic treatment generally not recommended!

Mellmann et al., Clin. Infect. Dis. 2005 Bielaszewska et al., PLoS ONE 2007 Friedrich et al., Clin. Infect. Dis. 2007 Bielaszewska et al., Clin. Infect. Dis. 2008

Adapted from Tarr et al., Lancet 2005



Enteroaggregative E. coli (EAggEC)





PhD Thesis

STATENS SERUM INSTITUT

Virulence Characterization and Epidemiology of Diarrheagenic Enteroaggregative *Escherichia coli* (EAEC)

> Nadia Boisen B.Sc., M.Sc. (Bio)





25 May 18:47



TESSy has reports of the below: TESSy 2007-2011

Serotype	eae	VT1	VT2	No HUS	HUS	NA	Reporting Country
104:H2	Neg	Neg	VT2	1		1	DK
104:H21	Unk	Unk	Unk		1		AT
104:H-	Neg	VT1	Neg	1	her	1	NO
104:HUNK/NT	Neg	VT1	Neg	2		1	NO
104:UNK/NT	eae	Neg	VT2			1	SE
Total		•		4	1	1	6 in total

Enternet had reports on 8 isolates: 2001-2006: No clinical data!!



Serotype	VT1	VT2	eae	Reporting Country	2001	2002	2004	2005	2006	Total
104:HND	neg	VT2	ND	NO	~	10		-	1	1
104:H21	VT1	VT2	neg	DE		1				1
104:H4	neg	VT2	neg	FR			1			1
104:H4	neg	VT2	neg	DE	2	1	1		Y	2
10 <mark>4:</mark> H7	VT1	neg	eae	DK	1.0		1			1
104:H7	VT1	neg	neg	AT				1		1
104:H7	VT1	neg	neg	DE	1					1
Total					3	1	2	1	1	8



ROBERT KOCH INSTITUT

EHEC O104:H4

Eigenschaften des Erregers sowie Hinweise und Hilfestellungen des RKI zur Diagnostik des gegenwärtig zirkulierenden Ausbruchstammes

(1. Veröffentlichung: 26.5.2011; Aktualisiert: 24.6.2011)

Eigenschaften des Erregers

Bei dem in dem gegenwärtigen Ausbruch (Mai/Juni 2011) zirkulierenden E. coli Stamm handelt es sich um einen pathogenen Vertreter von

E. coli Serovar O104:H4.

Er vereinigt Merkmale Shigatoxin-bildender E. coli und enteroaggregativer E. coli:

EHEC Merkmale:

- Shigatoxin 1: (negativ)
- Shigatoxin 2 (vtx2a) : + (positiv)
- Intimin (eae) : (negativ)
- Enterohämolysin : (negativ)

EaggEC Merkmale (EaggEC Virulenzplasmid):

- aatA-PCR: + (positiv) (ABC-transporter protein gene) aggR-PCR: + (positiv) (master regulator gene of virulence-plasmid genes) aap-PCR: + (positiv) (secreted protein dispersin gene) aggA-PCR: (AAF/I-fimbral subunit-gene) # + (positiv) (AAF/I-fimbral operon-gene) # aggC-PCR: + (positiv) astA-PCR (enteroaggr. E. coli heat-stable enterotoxin (EAST-1) gene) § - (negativ)
- # Unterschied zum O104:H4 Stamm HUSEC041 (www.ehec.org)/(RKI-01-09591), welcher Fimbrien des Typs AAF/III bildet (Prager R., Fruth A., and Tschäpe H., poster abstract XX, EHEC Workshop 2007).
- § Unterschied zum O104:H4 Stamm HUSEC041 (www.ehec.org)/(RKI-01-09591), welcher astA-PCR positiv ist.

Vergleichbare Stämme wurden bisher insgesamt selten und als solche primär beim Menschen nachgewiesen (s. Literatur am Ende des Beitrages).

MLST Sequenztyp: ST678 (adk 6, fumC6, gyrB 5, icd 136, mdh 9, purA 7, recA 7). (**)

26 May

Der Stamm weist folgende Antibiotikaresistenzen auf. (Wir weisen ausdrücklich darauf hin, dass diese Angaben nur der Charakterisierung des Stammes dienen und keine Indikation zu einer antimikrobiellen Therapie nahelegen sollen !)

R

R R

R*

R R

R

R

R

R

SSSSSSR

R

S S R

S

R

S S

Ampicillin Amoxicillin/Clavulansäure Piperacillin/Sulbactam Piperacillin/Tazobactam Cefuroxim Cefuroxim-Axetil Cefoxitin Cefotaxim Cetfazidim Cefpodoxim Imipenem Meropenem Amikacin Gentamicin Kanamycin Tobramycin Streptomycin Nalidixinsäure Ciprofloxacin Norfloxacin Tetracyclin Nitrofurantoin Trimethoprim/Sulfamethoxazol Chloramphenicol Fosfomycin

26 May





28 May 10:33

Four strains - two from Danish patients, one from a German female and one from Finland (se the Finnish report of 25-05 at 15:10 pm) are positive for aggR, aatA, aaiC and aap.

We consider these as aggregative.

All 4 strains are O104 and two have been confirmed as H4. They can be live slide agglutinated with K9 antiserum for quick identification because O104 is identical to K9. Further strain characteristics can be found at rki.de:

http://www.rki.de/cln_116/nn_217400/EN/Home/EHEC__0104__H4,templateId=raw,property=pu blicationFile.pdf/EHEC_0104_H4.pdf

SSI Diagnostica

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Use K9 serum to screen for E. coli O104

> Distributors

ADOUL SST Diagnostica

English > SSI Diagnostica > SSI Diagnostica news > 2011 > Use K9 serum to screen for E, coli O104

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30 May 2011

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V Ordening and snipping

> Web shop

The WHO Collaborating Centre for Reference and Research on Escherichia and Klebsiella at Statens Serum Institut suggests the use of K9 and O104 antisera for screening and confirmation of E. coli O104. Both O104 and K9 antisera are available from SSI Diagnostica

The bacterium E.coli O104:H4 has been identified as the cause of the ongoing outbreak in Northern Germany and belongs to the pathogenic group of E. coli which produces verocytotoxins (VT). VTEC strains can cause bloody diarrhoea and eventually lead to kidney failure in the form of haemolytic ureamic syndrome (HUS).

As of May 29, 15:00 CET, a total of 329 cases of haemolytic uraemic syndrome (HUS) was reported to the Robert Koch Institute (RKI) since the beginning of May 2011, including three deaths. Of all cases, 71% were female and 88% 20 years or older.



Identification of E. coli O104 can be done by means of serotyping with K and O antisera. An E, coli O104 strain will turn positive within 10 seconds when slide applutinating with K9 antiserum. Accordingly, K9 serum may be used to screen for E, coli O104 (for procedure - please see packaging insert for E. coli OK O antisera). A negative result will indicate that it is not an O104 strain. It is still recommended to confirm E. coli O104 with specific O antiserum following a positive slide applutination result.

Available products

Article number	Product	Packing
84888	E. coli K9 antiserum	1 mL vial
45840	E. coli 0104 antiserum	3 mL vial

DIAGNOSTICA

Search in Diagnostica:

Search

Søg

Contact

Statens Serum Institut SSI Diagnostica Herredsvejen 2 3400 Hillerød Denmark

Fax: +45 4829 9179 Tel: +45 4829 9178

microbiology@ssi.dk (inquiries) ivdorders@ssi.dk (ordering) www.ssi.dk





31 May 10:35

Dear Flemming We received four strains from your lab this morning. Thank you for this.

2. June 09:25

The real Time PCR procedure for the detection of the O104 serogroup gene works well...

Eurosurveillance





Europe's journal on infectious disease epidemiology, prevention and control

E-ALERT

Characteristics of the enteroaggregative Shiga toxin/ verotoxin-producing *Escherichia coli* O104:H4 strain causing the outbreak of haemolytic uraemic syndrome in Germany, May to June 2011

F Scheutz (fsc@ssi.dk)^{1,2}, E Møller Nielsen², J Frimodt-Møller^{1,3}, N Boisen^{1,2}, S Morabito⁴, R Tozzoli⁴, J P Nataro⁵, A Caprioli⁴

- World Health Organization Collaborating Centre for Reference and Research on Escherichia and Klebsiella, Department of Microbiological Surveillance and Research, Copenhagen, Denmark
- Food-borne pathogens and typing, Department of Microbiological Surveillance and Research, Statens Serum Institut, Copenhagen, Denmark
- 3. Department of Clinical Microbiology, Hillerød Sygehus, Hillerød, Denmark
- 4. European Union Reference Laboratory for Escherichia coli, Department of veterinary public health and food safety, Istituto Superiore di Sanità, Rome, Italy
- 5. University of Virginia School of Medicine, Charlottesville, United States



Typical E. coli

Sorbitol positive, < 24 hours

β-glucuronidase positive

Resistant for cefixime & tellurite







Previous findings of O104:H4

>90%	PFGE-Xbal	PFGE-BINI	Year Country	Pathotype
>90%	PFGE-Xbal	PFGE-BIni	Year Country 2011 Denmark 2011 Denmark 2011 Germany 2011 USA 2009 Rep. Georgia 2009 Mali 2010 Finland 2009 Rep. Georgia	Pathotype EAggEC-VTEC EAggEC-VTEC EAggEC-VTEC EAggEC-VTEC EAggEC EAggEC EAggEC
			2009 Mali 2009 Mali 2000 Denmark 2009 Mali 2001 C. African Rep. 2009 Mali 2009 Mali	EAggEC EAggEC EAggEC EAggEC EAggEC EAggEC EAggEC



Major findings

- the characterisation of an unusual combination of pathogenic features typical of EAggEC combined with the capacity to produce Vero cytotoxin in the outbreak strain;
- recommendations for simple diagnostic screening tools for primary laboratory detection of the outbreak strain in clinical specimens;
- a novel real-time PCR protocol for detection of *E. coli* O104:H4 in foods;
- presentation of the known occurrence and clinical presentation in humans and the likely reservoir.

Additional findings of O104







Recent French isolate from Turkey

PFGE Xbal	PFGE Xbal				
>90%	K	5			
-100	-700.00 -700.00 -500.00 -500.00 -260.00 -260.00 -160.00 -160.00 -160.00 -160.00 -160.00 -160.00 -160.00 -26.00				
		Ec11-4632.2	Bordeaux 2011	O104	H4
		Ec11-4623	Bordeaux 2011	0104	H4
	M 1 1800 0 1800 8 1 18 18	Ec11-4404	Bordeaux 2011	0104	H4
	B	Ec11-4984	Bordeaux 2011	0104	H4
		Ec11-4988	Bordeaux 2011	0104	H4
	Berner (1997) (1	Ec11-6006	Bordeaux 2011	0104	H4
		Ec11-3677	Germany 2011	0104	H4
	8 1 110 0 100 0 1 11 11 11 11	Ec11-3798	Germany 2011	0104	H4
		Ec11-4522	Germany 2011	0104	H4
		Ec11-5536	Bordeaux 2011	0104	H4
Г		Ec11-5537	Bordeaux 2011	0104	H4
		Ec11-5538	Bordeaux 2011	0104	H4
		Ec11-9450	Turkey 2011	0104	H4
		CRBIP14.5	Reference strain	0104	H4
	- I	Ec09-7901	France 2009	0104	H4
		Ec04-8351	France 2004	0104	H4
		CIP105999	Reference strain	0104	H21
		CIP106042	Reference strain	0104	H?
		H519	Reference strain	0104	H12

Courtesy of Francois-Xavier Weill, Institut Pasteur, Paris

The Lancet



23 June

Articles

Characterisation of the Escherichia coli strain associated with \Im an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study

Martina Bielaszewska, Alexander Mellmann, Wenlan Zhang, Robin Köck, Angelika Fruth, Andreas Bauwens, Georg Peters, Helge Karch

Summary

Background In an ongoing outbreak of haemolytic uraemic syndrome and bloody diarrhoea caused by a virulent *Escherichia coli* strain O104:H4 in Germany (with some cases elsewhere in Europe and North America), 810 cases of the syndrome and 39 deaths have occurred since the beginning of May, 2011. We analysed virulence profiles and relevant phenotypes of outbreak isolates recovered in our laboratory.

Methods We analysed stool samples from 80 patients that had been submitted to the National Consulting Laboratory for Haemolytic Uraemic Syndrome in Münster, Germany, between May 23 and June 2, 2011. Isolates were screened with standard PCR for virulence genes of Shiga-toxin-producing *E coli* and a newly developed multiplex PCR for

Published Online June 23, 2011 DOI:10.1016/51473-3099(11)70165-7

See Online/Comment DOI:10.1016/S1473-3099(11)70166-9

Institute for Hygiene and the National Consulting



Evolution of O104:H4



Figure 5. Evolutionary model of the origin of the German EHEC O104:H4 outbreak strain. Evolutionary model of the current outbreak strain (B226692) and the historical strain (01-09591) from 2001 based on whole chromosomal and plasmid data. Numbers on connecting lines indicate the number of loci that differ between the strains as determined by analysis of 1,144 core genome genes. The genes of the PCR test for differentiation of the HUSEC041 complex (str2: red; ter0: blue; rfb_{cr04}; green; fl/C_{H4}; yellow), [16] for antibiotic resistance (orange: TEM-1 and CTX-M-15), and for the differentiation of EAEC plasmids [5] (*stAt*: white) are colored. The order of plasmid acquisition and loss were arbitrarily chosen in the illustration as the exact sequence of events is not known. Year of isolation for each strain is noted in the lower left corner of each rectangle. doi:10.1371/journal.pone.0022751.g005

23 June



20 July

OPEN access Freely available online

PLos one

Prospective Genomic Characterization of the German Enterohemorrhagic *Escherichia coli* O104:H4 Outbreak by Rapid Next Generation Sequencing Technology

Alexander Mellmann¹*, Dag Harmsen²**, Craig A. Cummings³*, Emily B. Zentz⁴, Shana R. Leopold¹, Alain Rico⁵, Karola Prior², Rafael Szczepanowski², Yongmei Ji³, Wenlan Zhang¹, Stephen F. McLaughlin³, John K. Henkhaus⁴, Benjamin Leopold¹, Martina Bielaszewska¹, Rita Prager⁶, Pius M. Brzoska³, Richard L. Moore⁴, Simone Guenther⁵, Jonathan M. Rothberg⁷, Helge Karch¹

Institute of Hygiene, University Münster, Munster, Germany, 2 Department of Periodontology, University Münster, Germany, 3 Life Technologies, Foster City, California, United States of America, 4 OpGen, Gaithersburg, Maryland, United States of America, 5 Life Technologies, Darmstadt, Germany, 6 Robert Koch Institute, Werrigerode Branch, Wernigerode, Germany, 7 Jion Torrent by Life Technologies, Guilford, Connecticut, United States of America



ORIGINAL ARTICLE



Comparison of the outbreak strain Origins of the E. coli Strain Causing an Outbreak and EAggEC 0104:H4 from Mali 27 July

David A. Rasko, Ph.D., Dale R. Webster, Ph.D., Jason W. Sahl, Ph.D., Ali Bashir, Ph.D., Nadia Boisen, Ph.D., Flemming Scheutz, Ph.D.,

of Hemolytic–Uremic Syndrome in Germany





The NEW ENGLAND JOURNAL of MEDICINE

The NEW ENGLAND JOURNAL of MEDICINE

ORIGINAL ARTICLE

Origins of the *E. coli* Strain Causing an Outbreak of Hemolytic–Uremic Syndrome in Germany

David A. Rasko, Ph.D., Dale R. Webster, Ph.D., Jason W. Sahl, Ph.D., Ali Bashir, Ph.D., Nadia Boisen, Ph.D., Flemming Scheutz, Ph.D.,









The cucumber lead





Hamburger Institut für Hygiene und Umwelt identifiziert Salatgurken als EHEC-Träger

Empfehlen

66 HUS-Erkrankungen in Hamburg - Verdacht auf O104 im EHEC-Stamm durch HU bestätigt

26.05.2011

+1 < 0



Agarmedium mit EHEC-Kolonien (Bild: HU)

> Twittern

0

Behörde für Gesundheit und Verbraucherschutz



C DETTE billede er taget den 26. maj på et marked i Hamborg. Agurkerne er spanske. Disse og alle andre agurker fra Spanien er nu fjernet fra markeder og butikshylder. - Foto: MARIUS ROEER/AP



Source of the outbreak

Cucumbers positive for VTEC Cucumbers negative for O104 No VTEC in Danish cucumbers 26 May 30 May 31 May



ROBERT KOCH INSTITUT



All 31 case subjects had been served a meal that contained sprouts. The result of the univariate analysis was confirmed in the multivariate analysis (RR: 14.2, 95% CI 2.4 - ∞ , p<0.01). The use of other raw ingredients such as tomatoes, cucumbers or green salad in the served meals showed no significant p values (p> 0.15) in the analysis for an increased relative risk of disease.

Table 3: Results of univariate and multivariate data analyses of the restaurant recipe-based cohort study. RR, relative risk; 95% CI, 95% confidence interval (CI).

Ingredient	Total	Cases among the exposed	Total number	Cases among the non-	Total number of	RR	95% CI	P-value
			exposed	exposed	non-exposed			
Univariate								
Sprouts	152	31	115	0	37	14.23	2.55-infinity	<0.01
Tomatoes	152	14	50	17	102	1.68	0.77-3.62	0.18
Cucumbers	152	14	50	17	102	1.68	0.77-3.62	0.18
Chinese	152	13	45	18	107	1.72	0.77-3.71	0.17
cabbage								
Radicchio	152	13	45	18	107	1.72	0.77-3.71	0.17
Iceberg	152	13	45	18	107	1.72	0.77-3.71	0.17
lettuce								
Multivariate								
Sprouts						14.17	2.40-infinity	<0.01

In the period in which the group dined in the restaurant, only one mixture of sprouts was used, containing fenugreek sprouts, alfalfa sprouts, adzuki bean sprouts and lentil sprouts. The supplier of the restaurant supplying the sprouts received his goods from the incriminated company A in Lower Saxony.



Source of the outbreak

Cucumbers negative for O104 No VTEC in Danish cucumbers Sprouts suspected Sprouts the main suspected source French outbreak EFSA report

30. May
31. May
7 June
10 June
24 June
5 July



Supporting publications

Corporate publications

Tracing seeds, in particular fenugreek (Trigonella foenum-graecum) seeds, in relation to the Shiga toxin-producing E. coli (STEC) O104:H4 2011 Outbreaks in Germany and France



Task force conclusion

Same supplier of seeds for sprouting in France and to the German sprouting producer in Lower Saxony leads to the conclusion that Fenu Greek seeds imported from Egypt in 2009 was the source.



Trigonella foenum-graecum



EHEC report RKI

Figure 1 shows the epidemiological curve of HUS and EHEC cases. Both curves sharply rise from 8 May on, peak around 22 May and then decline. The latest date of disease onset was contracted is 23 June. Whether or not cases with disease onset prior to 8 May, belong to the outbreak, cannot yet be assessed conclusively.





Lessons learned

- Inadequate diagnostic methods delayed detection of the outbreak strain
- The cucumber lead sidetracked investigations
- Notifications to RKI were delayed
- The incubation period was longer
- Sprouts are used in many ways eg in sandwiches and as topping
- Unusual and virulent *E. coli* pathotypes may arise at any time
- Research and international collaboration is essential in the investigations of food-borne outbreaks
- The networks worked well

New paradigm



New risk assesment of VTEC

	ATENS Læshø RUM STITUT	jt Kontakt Om S	SI Selvbetjening	<u>Svadomsleksikon</u>	Sitemap Er	nglish Søg	Q
Aktuelt	Smitteberedskab	Diagnostik	Vaccination	Forskning	Produkter	r og ydelser	Bestil
Forside > Sygdomsleksikon > H > Hæmolytisk uræmisk syndrom						Søg i Sygdoms	leksikon
Hæmo	olytisk uræm			Søg			

HUS associated VTEC

These virulence profiles have clinical relevant association with HUS;

- 1. vtx1 and eae and O103:H2;
- 2. vtx1 and vtx2 and eae;
- 3. vtx2 and eae;
- vtx2 in an enteroaggregative *E. coli* (EAggEC) eg. 0104 or 0111.
- 5. vtx2d in eae negative VTEC.

generelle dødelighed for HUS 90 - 100 %.



National reference laboratories – primary diagnostic laboratories



Also from us at SSI!







Susanne Jespersen

Pia Møller Hansen



Charlotte Hoe



Tyra Grove Krause

Steen Ethelberg



Eva Møller Nielsen

Nadia Boisen



Flemming Scheutz



Jakob Frimodt-Møller

Andreas Munk Petersen

Karen Krogfelt



Carsten Struve



Luise Müller

Kåre Mølbak

Charlotte Kjelsø

Maj-Britt Winther

Steffen Offersen Glismann