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Still culturing for gastrointestinal pathogens in your lab? What might you be missing?

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Conflicts of interest:

Franz Allerberger **:** Senior author of the *European, multi-centre, prospective quarterly point prevalence study of community acquired diarrhoea (EUCODI)*: supported by the Food- and Water-borne Infections Study Group (EFWISG) of the European Society of Clinical Microbiology and Infectious Diseases. BioFire Diagnostics (Salt Lake City, Utah) funded the study coordination plus sample transportation and provided test kits and instruments for laboratory analysis.

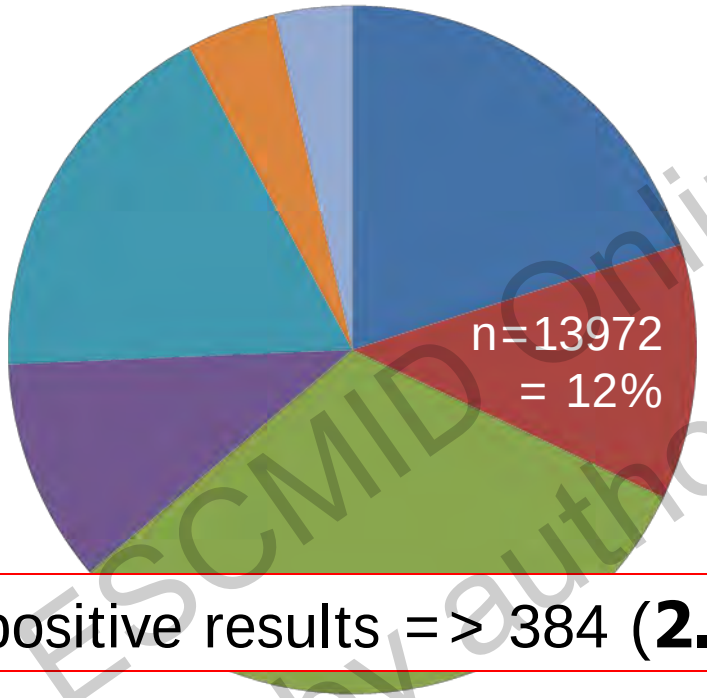
Katharina E. P. Olsen **:**

co-author of EUCODI,

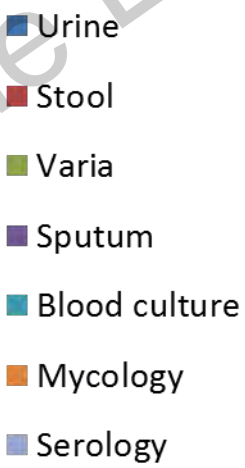
otherwise: nothing to declare

Specimen distribution, Austria 2014

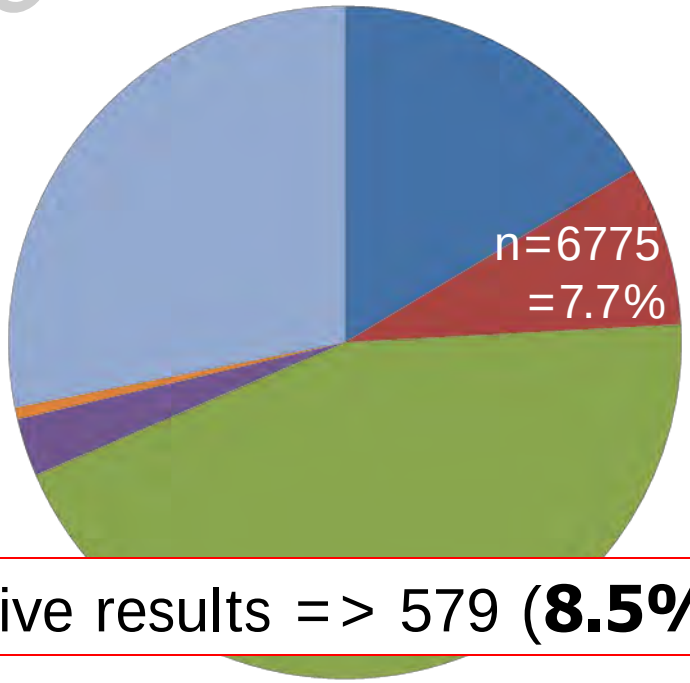
Hospital lab. N = 116401



positive results => 384 (**2.7%**)



Private outpatient lab. N = 87646



positive results => 579 (**8.5%**)

Among 230 prospecitvely collected samples routine testing was positive for one or more GI pathogens in 19 (**8.3%)** samples. Khare R et al. J Clin Microbiol 2014; 52:3667-73.

Huhulescu S, Kiss R, Brettlecker M, Cerny RJ, Hess C, Wewalka G, Allerberger F (2009) Etiology of acute gastroenteritis in three sentinel general practices, Austria 2007. *Infection* 37:103-108

We studied the etiology of acute gastroenteritis in a village with a total population of appr. 6,000. In 2007, all patients who visitied one of three local general practitioners for acute gastroenteritis were invited to provide stool specimens.

Pathogenes were detected in 71 of 306 patients (**23.2%**).

12 pathogens

Devices With GI Panels In Development Or FDA Clearance Process

22 pathogens

CLEARED

BD Max

CLEARED

BioFire FilmArray

GenMark Dx eSensor XT-8

Cepheid GeneXpert

In Clearance

Nanosphere's Verigene Enteric Pathogens (EP) Test

Applied Biocode

CLEARED

CLEARED

Luminex xTag GPP

Slide graduity: Jan Vinje

F

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WHICH GI PATHOGENS TO TEST FOR IN DAILY ROUTINE?

AND WHICH PATIENTS?

https://clinmicro.asm.org/images/Cumitechs/Cumitech12A.pdf

CUMITECH 12A Laboratory Diagnosis of Bacterial Diarrhea **1992**

Guidelines for outpatients:

• Screen for *Salmonella*, *Shigella*, and *Campylobacter* spp.

• In **certain geographic locales**, vibrios (along the coast of the Gulf of Mexico), *E. coli* O157:H7 (northwestern United States and Canada), and *Y. enterocolitica* or ETEC (areas with high numbers of migrant workers from Mexico and Central America).

• Patient who currently is receiving or recently has received **antimicrobial agents**, *C. difficile* must be considered

• *E. coli* O157:H7 in all patients in areas with **high endemicity** and in patients with frankly **bloody stools** in areas of low endemicity.

• People who consume **raw shellfish** are at increased risk for *Vibrio* infections.

• Children who have **chronic malabsorptive diarrhea** may be infected with *G. lamblia* or enteroadherent *E. coli*.

• In the **winter months**, children should first be screened for rotavirus, and only if that test is negative, testing for bacterial pathogens."

FilmArray GI ®Panel

(BioFire Diagnostics, Salt Lake City, Utah).

The FilmArray integrates sample preparation, amplification, detection, and analysis into one simple system that requires approximately 2 minutes of hand-on time and has a total run time of 1 hour. It allows the simultaneous detection of the following 22 targets:

Bacteria: *Campylobacter*, *Clostridium difficile*, *Plesiomonas shigelloides*, *Salmonella*, *Vibrio*, (including *V. cholerae*), *Yersinia enterocolitica*, Enterotoxigenic *Escherichia coli* (ETEC; *lt*/*st*), Enteropathogenic *E. coli* (EPEC), Shiga toxin-producing *E. coli* /STEC; *stx1*/*stx2*, including *E. coli* O157), Shigella/Enteroinvasive *E. coli* (EIEC), Enteroaggregative *E. coli* (EAEC),

Viruses: Adenovirus F40/41, Astrovirus, Norovirus GI/GII, Rotavirus A, Sapovirus

Protozoa: *Cryptosporidium*, *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia lamblia*

Detection of at least one organism:

22 GI pathogens

Spina A et al. Spectrum of enteropathogens detected by FilmArray® GI Panel in a multi-centre study of community-acquired gastroenteritis. Clin Microbiol Infect [2015] ;20. April; Epub ahead of print doi:10.1016/j.cmi.2015.04.007

Pathogens were detected in 384/709 patients (**54.2%**), as compared to 18.1% (128/709) when tested by conventional techniques locally.

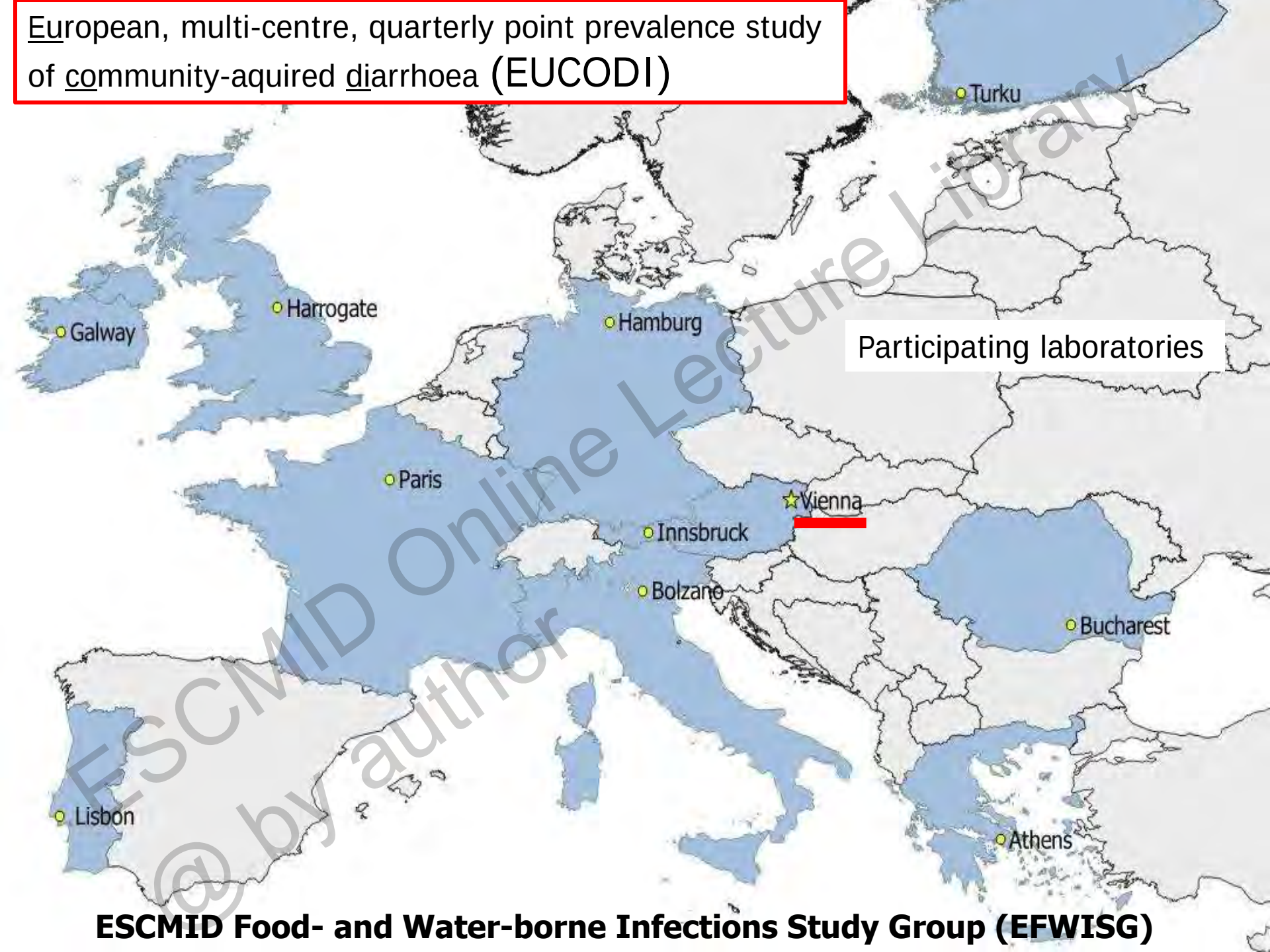
Buss SN et al. Multicenter Evaluation of the BioFire FilmArray™ Gastrointestinal Panel for the Etiolog Diagnosis of Infectious Gastroenteritis. J Clin Microbiol 2015; 53:915-25.

Path. were detected in 832/1556 patients (**53.5%**).

Khare R et al. Multiplex detection of gastrointestinal pathogens: A comparative evaluation of two commercial panels using clinical stool specimens. J Clin Microbiol 2014; 52:3667-73.

Path. were detected in 76/230 patients (**33.0%**), compared to 69 (30.3%) by Luminex assay and 19 (8.3%) by routine testing.

European, multi-centre, quarterly point prevalence study of community-aquired diarrhoea (EUCODI)

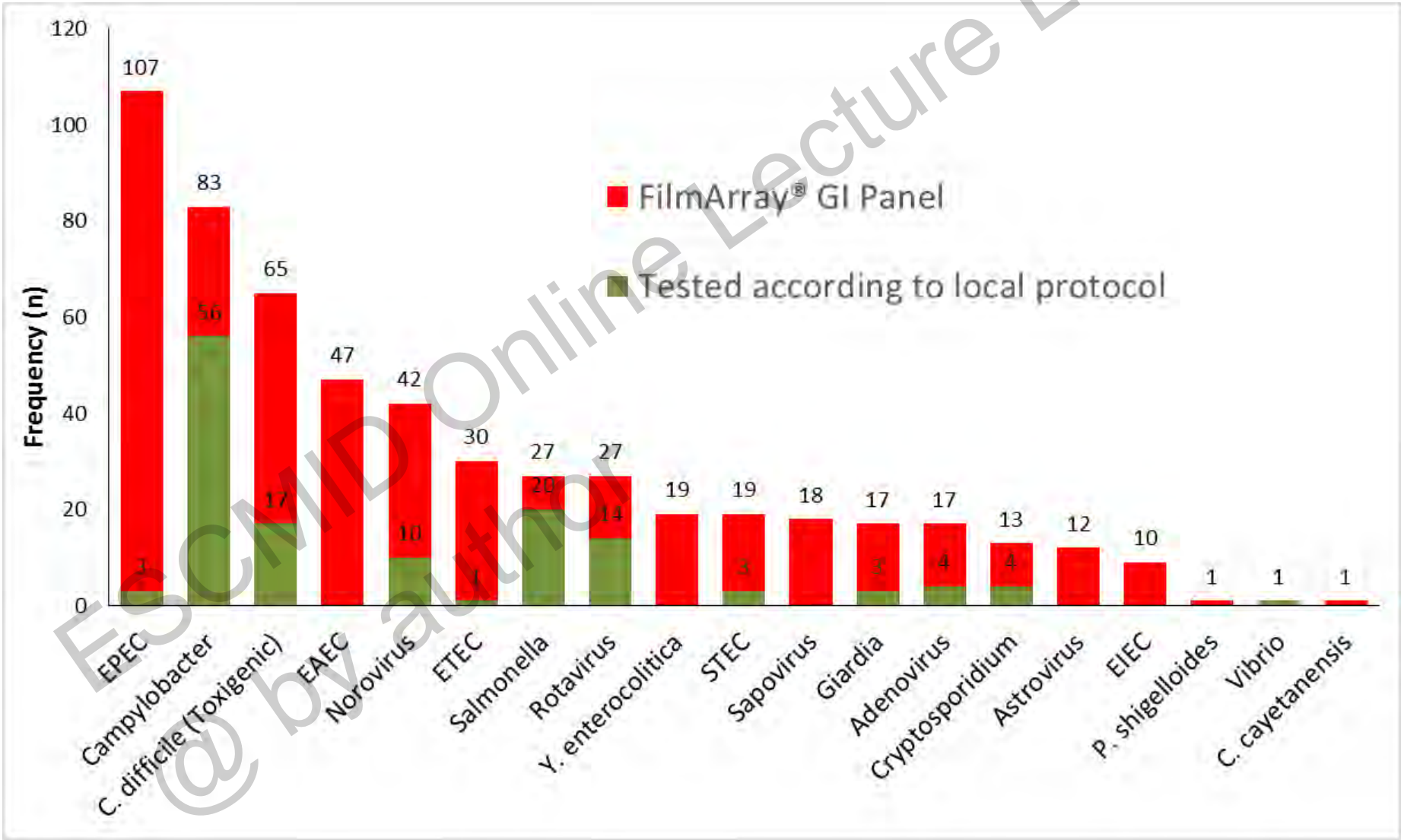


ESCMID Food- and Water-borne Infections Study Group (EFWISG)

- On four specified days, each local laboratory submitted samples from ≤20 consecutive patients to the Austrian Study Centre for further testing using FilmArray® GI Panel.
- Of the 709 specimens from as many patients received, 325 (45.8%) tested negative, 268 (37.8%) specimens yielded only one organism, and 116 (16.4%) specimens yielded multiple organisms. Positivity rates ranged from 41% (30/73 samples) in France to 74% (59/80 samples) in Romania.
- **With the exception of *Entamoeba histolytica* and *Vibrio cholerae*, all of the 22 targeted pathogens were detected at least once.**

54.2%

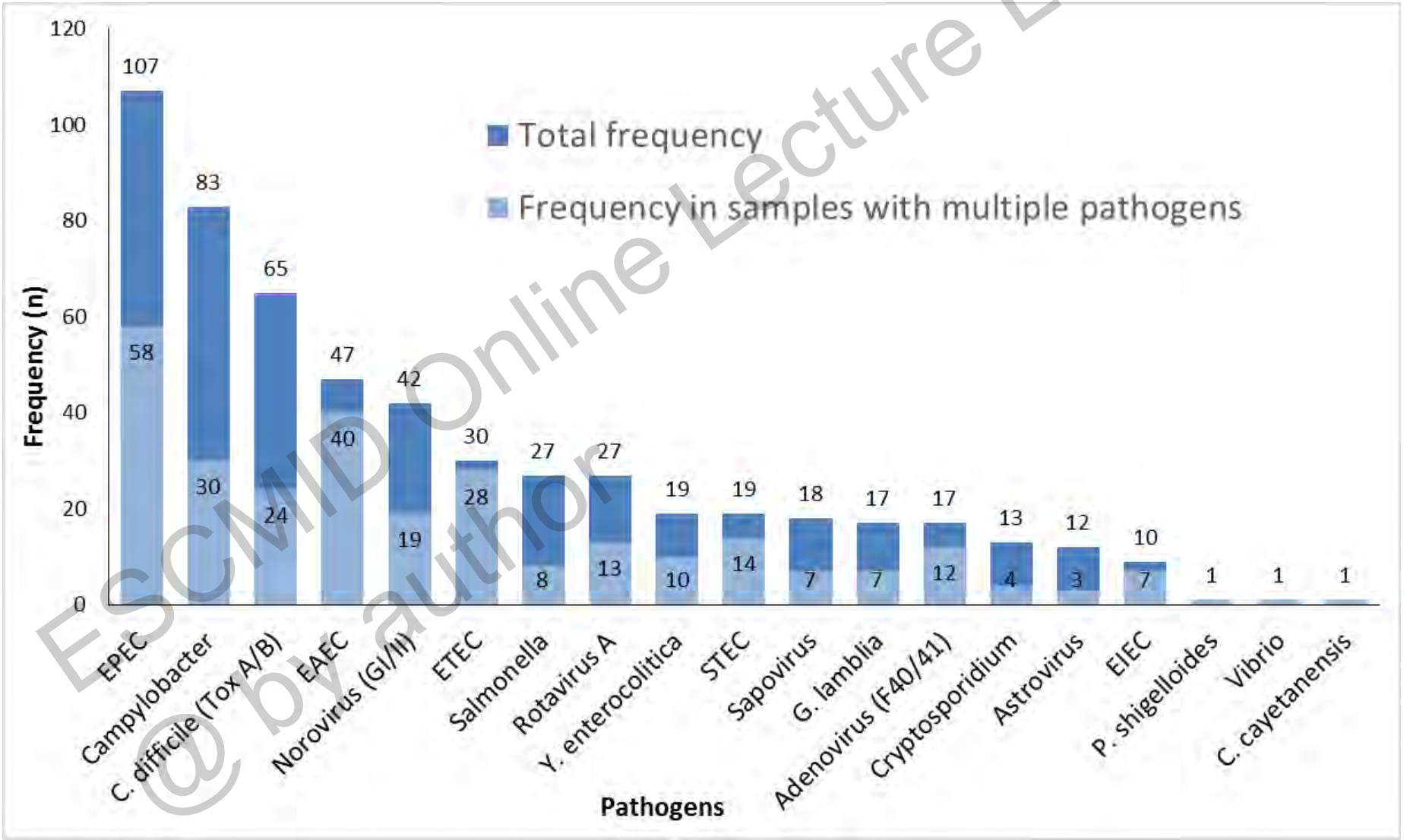
Fig.: Frequency distribution of pathogens detected in 709 stool specimens by diagnostic approach employed: local laboratory protocol versus FilmArray GI®Panel



Diagnostic approach employed by local laboratories

	EPEC	EAEC
Austria	No	No
Finland	No	No
France	No	No
Germany	Yes (eae gene PCR)	Yes (PCR)
Greece	No	No
Ireland	No	No
Italy	Yes (eae gene PCR)	No
Portugal	No	No
Romania	Yes (agar antisera)	No
UK	No	No

Figure 2: Overall frequency distribution of pathogens detected and frequency distribution of pathogens which were detected in a sample with multiple pathogens detected (total pathogens = 555)



Spina A. et al. (2015) Spectrum of enteropathogens detected by FilmArray® GI Panels in a multi-centre study of community-acquired gastroenteritis. *Clin Microb Infect* *20. April; Epub ahead of print*
doi:10.1016/j.cmi.2015.04.007

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"The FilmArray GI Panel reported a total of 181 (17%) specimens with multiple organism detections (i.e., mixed infections), which represents **31%** (118/384) of positive specimens. EPEC or EAEC were present in 98 of 116 (84%) of samples with multiple pathogens detected**, which raises the question of the clinical relevance of these pathogens,** which were not routinely tested for in the participating laboratories. Indeed 85% of EAEC positive samples and 54% of EPEC positive samples contained other pathogens."

Buss SN et al. J Clin Microbiol 2015;53:915-25.
Mixed infections in **31.1%** of positive samples

Khare R et al. J Clin Microbiol 2014;52:3667-73.
Mixed infections in **21.1%** of positive samples (8.3% by routine methods).

K

EPEC and EAEC: State of the art

EPEC and A/EEC: How to discriminate

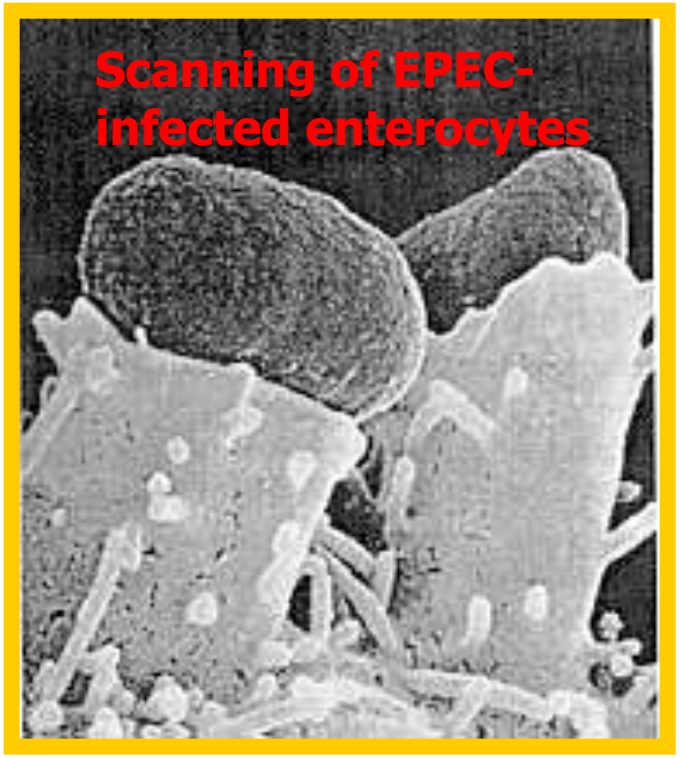
Symptoms: Often persistent watery diarrhoea (commonly without blood) incl. vomiting. Fever and nausea. Colonize especially the small intestine.

EPEC is the most prevalent bacterial cause of diarrhoea for infants < 2 år.

A/EEC is often from patients with persistent diarrhoea.

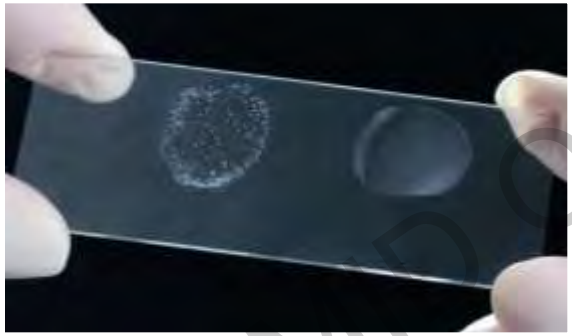
Virulence genes: ***eae*** on the locus of enterocyte effacement (= LEE)

Histopathology: Attach in bundles leading to actin condensation and microvillous effacement



Classical EPEC O:H serotypes

PCR/probe-positive colonies: live slide agglutination with O antisera against the most common EPEC O-groups:



A/EEC or EPEC

O26	H-; H11; H34
O55	H-; H6;H7
O86	H-; H34
O111	H-; H2; H25
O114	H-; H2
O119	H-; H2; H6
O125ac	H-; H6;H21
O126	H-; H2; H21; H27
O127	H-; H6; H21
O128ab	H-; H2; H7; H12
O142	H-; H6
O158	H-; H23

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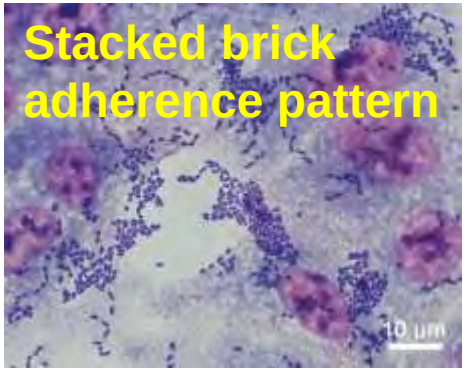
Infant-diarrhoea project

Case-control study of infants <5 yrs (year 2000-2001)

Bacteria	Cases (%) n=219	Controls (%) n=416	*p*
Campylobacter	11(5)	3 (<1)	0.002
Salmonella	8 (4)	2 (<1)	0.0081
EPEC	7 (3)	2 (<1)	0.02
STEC	6 (3)	2 (<1)	0.04
Yersinia	6 (3)	1 (<1)	0.016
Shigella	0	0	-
ETEC	3 (1)	6 (1)	1
EAEC	5 (2)	5 (1)	0.5
A/EEC	26 (12)	43 (10)	0.6

Olesen B. et al., JCM (2005)

Enteroaggregative E. coli (EAEC)



Symptoms: Watery secretory diarrhoea often with mucus. Low-grade fever, nausea, vomiting, abdominal pain and occasionally bloddy stool . Persistent diarrhoea in children < 1 yr of age in endemic areas.
Incubation period ranges from 8 h to 52 h.

Travelers diarrhoea.

Virulence genes: ***aatA*** (plasmid), ***aaiC*** (chromosome) and ***aggR*** (encoded on pAA virulence plasmid). *

* *aggR*, AAF/I-AAF/IV genes, *aap, aatA, fis, shf, yafK, astA, pet, sepA, sat, set pic etc.*

Due to genetic heterogeneity of EAEC geographically the combination of virulence genes for diagnostic purposes have to vary!

Jensen BH. et al. CMR (2014)

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Other limitations of molecular screening for GI pathogens?

Do we have access to the gene targets from the manufacturer of the molecular assays for the purpose of

- risk assessment of severity of disease?
- judging the comprehensiveness of the GI panel?

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What about *Aeromonas*?

A preliminary study at SSI showed that 79% of the *A. hydrophila* strains carried two hemolysin genes (*ahh1* and *hlyA*) and two enterotoxin genes (*alt* and *ast*) rendering this species of particular high pathogenic potential.

Genes	*A.hydrophila* N=28 (%)	*A.veronii* N=71 (%)	*A.caviae* N=101(%)	*A.media* N=31 (%)
aerA	17 (61)	0	0	0
asa1	1 (4)	65 (92)	1 (1)	0
ahh1	26 (93)	0	0	0
hlyA	25 (89)	0	0	1 (3)
act	5 (18)	64 (90)	0	2 (6)
alt	27 (96)	7 (10)	100 (99)	27 (87)
ast	23 (82)	0	0	0

Yersinia enterocolitica biotype 1A?

Biotype 1A is considered avirulent because of lack of the pYV virulence plasmid and major chromosomal virulence genes: *Ail* adhesin, *YstA* enterotoxin, and the *Ysa* type 3 secretion system.

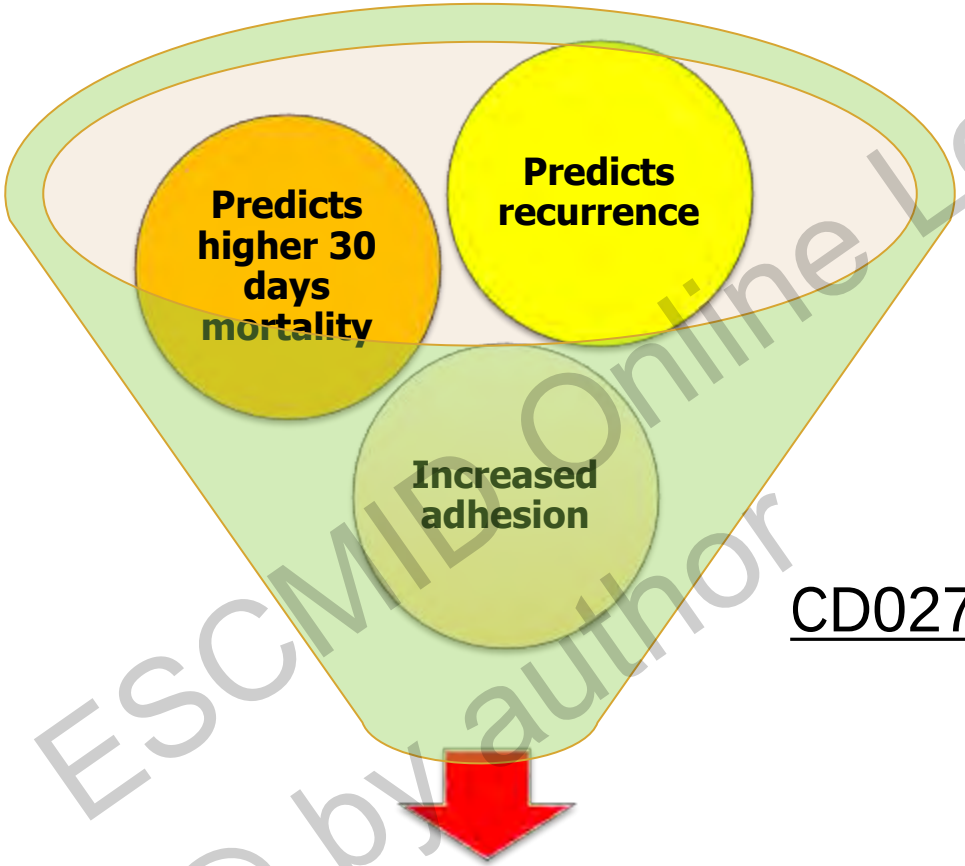
However, biotye 1A strains can evoke disease symptoms indistinguishable from those produced by the pathogenic biotypes.

In a Finnish study imported fruits and berries were associated with increased risk of *Y. ent.* biotype 1A - OR 3.5 (1.2-10.5).

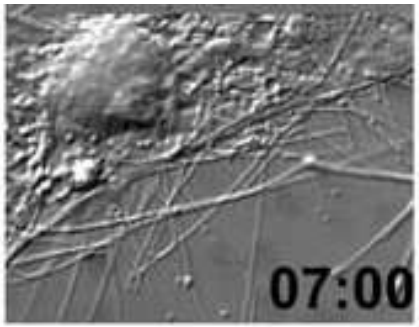
YstB as a diagnostic marker for *Y. enterocolitica* biotype 1A?

Huovinen E. et al., BMC BMC Infect Dis (2010)
Bhagat N. et al. Critical Reviews in Microbiology (2011)

Binary toxin plays a central role in risk assessment



- **Choice of antibiotics**
- **Isolation of patients**



Caco-2 cells and binary toxin (Scwan et al.)

CD027 is binary toxin positive!

Schwan, PLoS Pathogens (2009); Stewart, J Gastrointest. Surg. (2012); Bacci, EID (2011).

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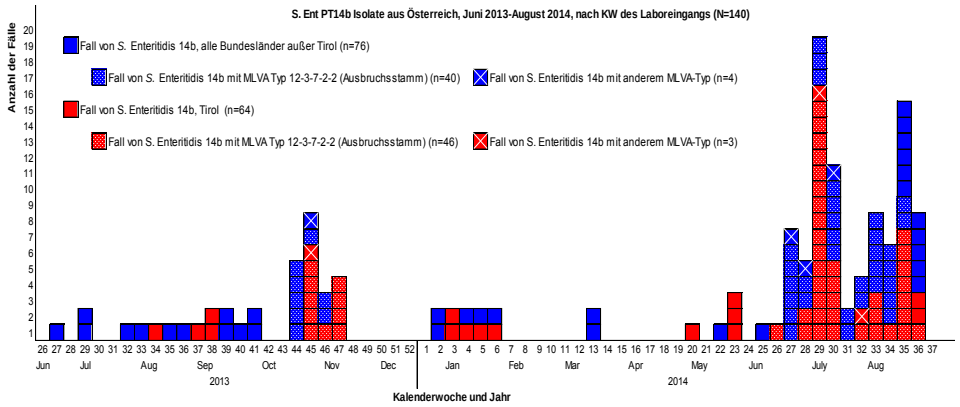
Salmonella- and STEC-diagnosis (*and in-vitro susceptibility testing*) still ask for isolates



Time Distribution, S. *Enteritidis* PT14b-outbreak strain and non-outbreak strain, Austria, July-Sept. 2014

Regional Distribution, S. *Enteritidis* PT14b-outbreak strain, Austria, July-Sept. 2014

efsa European Food Safety Authority

ecdc

EFSA supporting publication 2014:EN-646

Issued: 26 August 2014

TECHNICAL REPORT

Multi-country outbreak of *Salmonella* Enteritidis infections associated with consumption of eggs from Germany[1]

European Centre for Disease Prevention and Control

European Food Safety Authority[2]

European Centre for Disease Prevention and Control (ECDC), Stockholm, Sweden

European Food Safety Authority (EFSA), Parma, Italy

Multi-country outbreak of Salmonella Enteritidis infections associated with consumption of eggs from Germany – 25 August 2014. Stockholm and Parma: ECDC/EFSA; 2014

European Food Safety Authority

TECHNICAL REPORT OF EFSA

April-July 2011: 2987 cases of gastroenteritis, 855 cases of HUS, 53 fatalities

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[1]

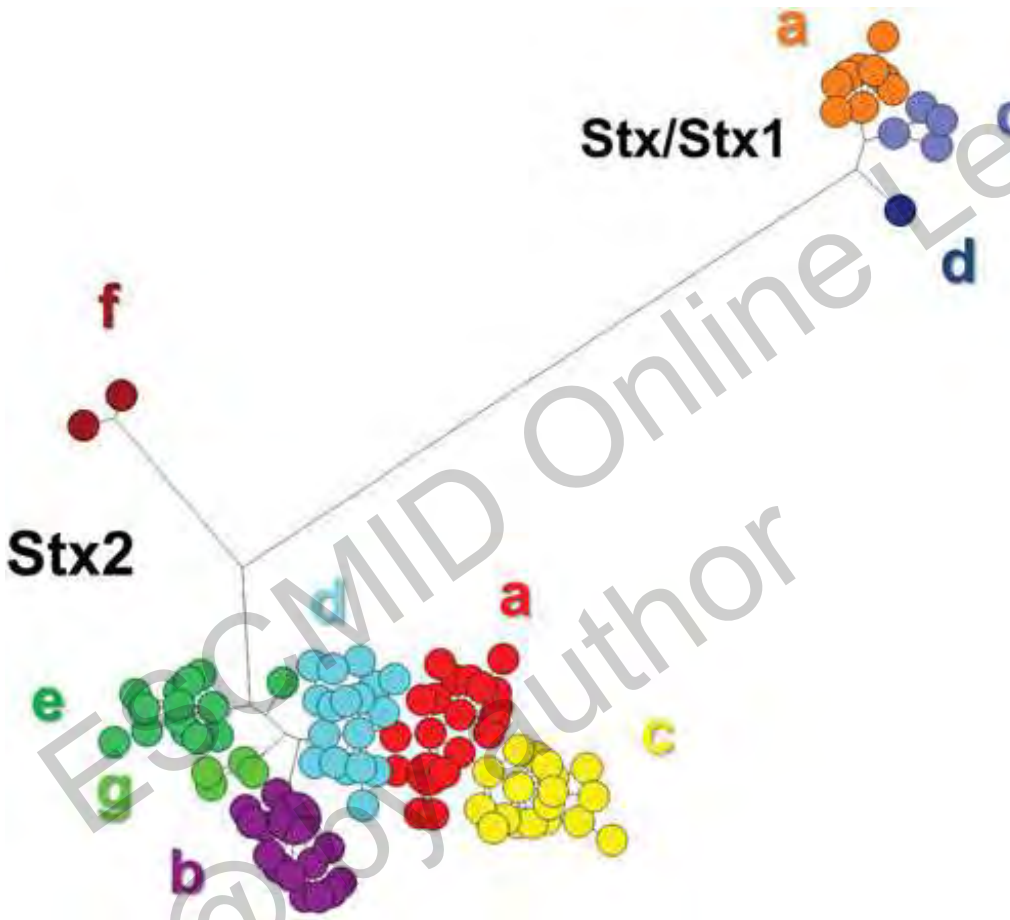
European Food Safety Authority[2, 3]

European Food Safety Authority (EFSA), Parma, Italy

uropean Food Safety Authority. Tracing seeds, in particular fenugreek (*Trigonella foenum-graecum*) seeds, in elation to the Shiga toxin-producing *E. coli* STEC O104:H4 outbreaks in Germany and France. Parma, Italy: uropean Food Safety Authority; 2011. Available at http://www.efsa.europa.eu/en/supporting/doc/176e.pdf

KEY WORDS

Sequence-based subtyping of Stx1 and Stx2



Scheutz et al., JCM (2012): 2951-2963.

Stx2a (with or without Stx2c) is highly associated with HUS.
Ref.: Kawano et al., Eur J Clin Microbiol Infect Dis (2008): 227-32.

An association between the activatable subtype Stx2d in eae-negative STEC and HUS has been found.
Ref.: Bielaszewská M et al., Clin Infect Dis (2006): 1160-1167.

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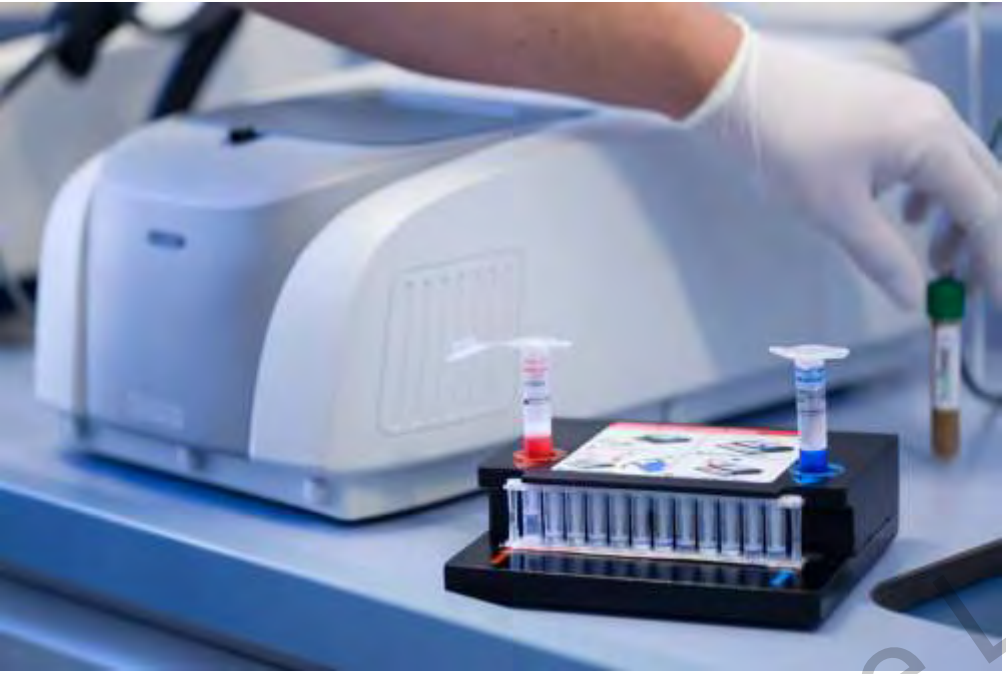
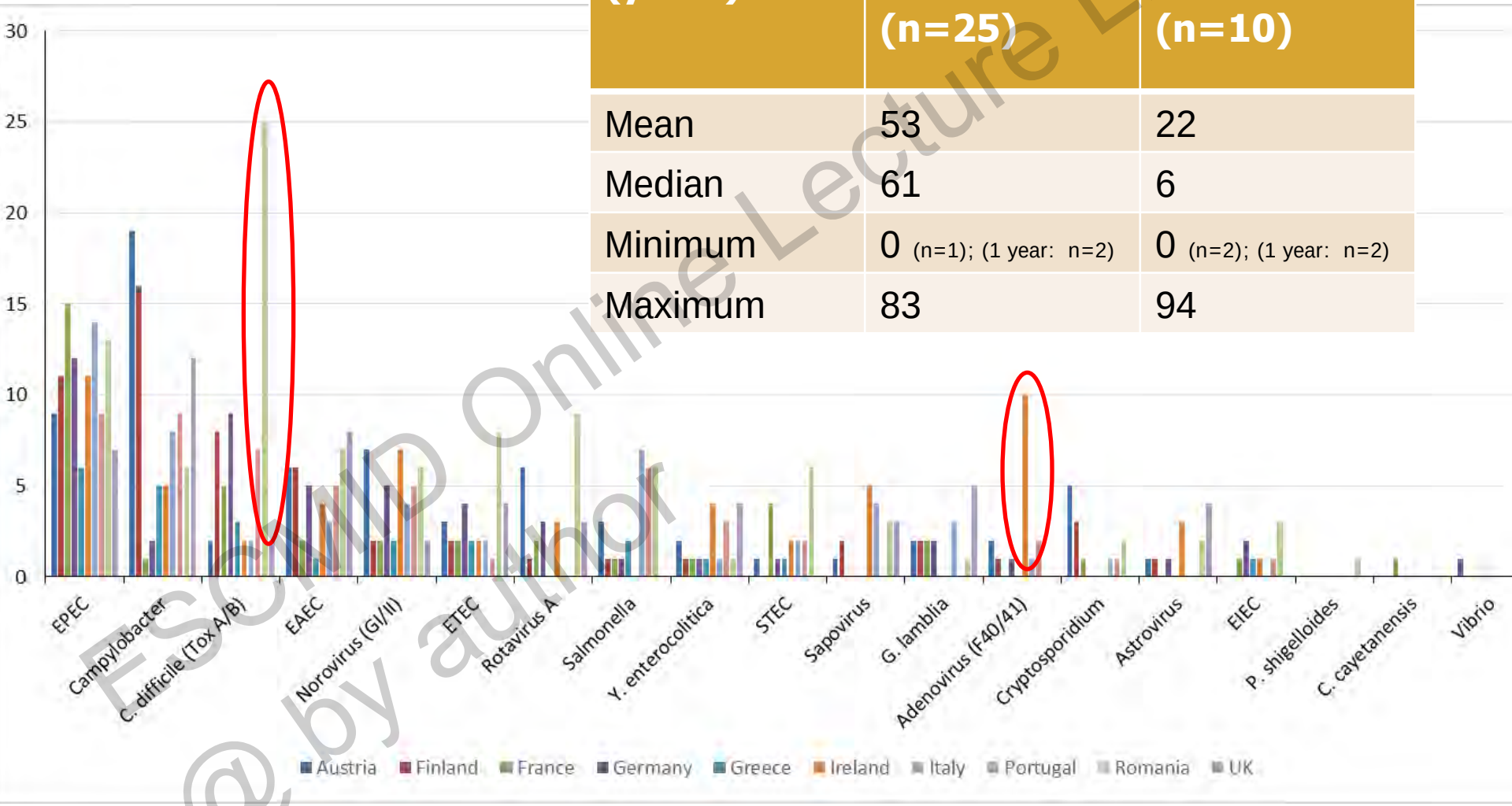
Multiplex PCR-screening tests speed up diagnosis and extend the test-spectrum, however they are complementing but not abolishing classical methods.



The **FilmArray** integrates sample preparation, amplification, detection, and analysis into one simple system that requires approximately 2 minutes of hand-on time and has **a total run time of 1 hour.**

Fig.: Frequency distribution of pathogens detected by country (total pathogens = 555)

Age (years)	C. difficile Romania (n=25)	Adenovirus Ireland (n=10)
Mean	53	22
Median	61	6
Minimum	0 (n=1); (1 year: n=2)	0 (n=2); (1 year: n=2)
Maximum	83	94



Diagnostic spectrum and **timeliness** directly influence risk assessment in foodborne infection and in nosocomial infection control.

I predict that NGS (next generation sequencing) will reach the routine laboratory within the next few years.

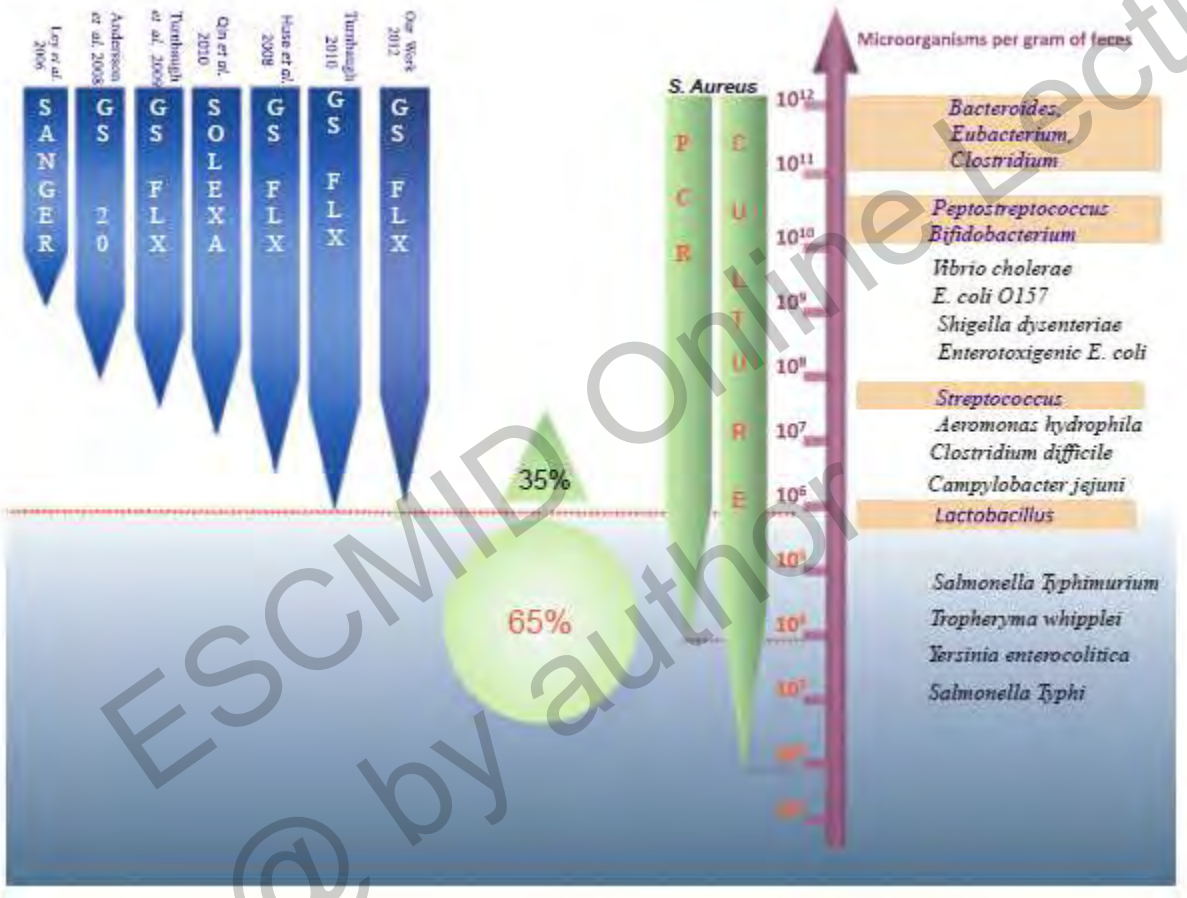
query for Katharina:

Will NGS abolish classical culture?

K

Even NGS will not abolish classical culture!

Detection thresholds of metagenomic and culturomic approaches



"The depht bias"

	16S rRNA	Culture
Species	282	340
Phyla	6	7
Genera	91	117

Lagier JC et al., CMI (2012)

THANKS TO ALL STUDY-PARTICIPANTS

European, multi-centre, quarterly point prevalence study of community-aquired diarrhoea (EUCODI)

Alex SPINA[1], Kevin KERR[2], Martin CORMICAN[3], Frédéric BARBUT[4], Angelika EIGENTLER[5], Loukia ZERVA[6], Panayotis TASSIOS[6], Gabriel POPESCU[7], Alexandru RAFILA[7], Erkki EEROLA[8], Judite BATISTA[9], Matthias MAASS[10], Richard ASCHBACHER[11], Katharian OLSEN[12], Franz ALLERBERGER[1]

[1]Austrian Agency for Health and Food Safety, Vienna, AT
[2]Harrogate District Hospital, Harrogate, GB
[3]National University of Ireland, Galway, IE
[4]Hôpital Saint-Antoine, Paris, FR
[5]Labor Moest, Innsbruck, AT
[6]Attikon University Hospital, Athens-Chaidari, GR
[7]National Institute for Infectious Diseases, Bucharest, RO
[8]Turku University, Turku, FI
[9]Occidental Hospital Centre, Lisbon, PT
[10]Labor Dr. Heidrich & Kollegen MVZ, Hamburg, DE
[11]Laboratorio Aziendale di Microbiologia e Virologia Bolzano, Bolzano, IT
[12]Statens Serum Institut, Copenhagen, DN

Spectrum of enteropathogens detected by FilmArray® GI Panels in a multi-centre study of community-acquired gastroenteritis. *Clin Microbiol Infect [2015] ;20. April; Epub ahead of print doi:10.1016/j.cmi.2015.04.007*

- END -

Thank you for attending
the Meet the Expert Session

Still culturing for gastrointestinal pathogens in your lab?
What might you be missing?

Franz Allerberger and *Katharina E. P. Olsen*

Please fill out the assessment sheets.

ESCMID EUROPEAN SOCIETY OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES

ESCMID Food- and Water-borne Infections Study Group – EFWISG