Educational Workshop

EW08: What's new on the menu? Emerging issues in food-borne infection

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

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Emerging issues in food-borne infection

‘We never look for it because we never find it’ – emerging pathogens we need to know about

John Threlfall
Health Protection Agency, UK

ECCMID April 2013

‘There are known knowns; there are things we know we know. We also know there are known unknowns; that is to say, we know there are some things we do not know’.

‘But there are also unknown unknowns – the ones we don’t know we don’t know’.

Donald Rumsfeld, US Defence Secretary, 2002

‘We never look for it because we never find it’ – emerging pathogens we need to know about

Disease
• What are the symptoms?
• What is the causative organism?
• How rapidly is this isolated?
• How common is the organism? – individuals, communities

Source / reservoir
• Is it a food contamination issue?
• If so, what is the food?
• What is the animal reservoir (if any?)
Threlfall - We never look for it because we never find it; emerging food-borne pathogens you need to know about

Organisms / Disease

- Listeriosis
- Vector-borne salmonellosis
- Infant and food-borne botulism
- Verotoxigenic *Escherichia coli*
- Hepatitis E (HEV)

Listeriosis
Organism
- *Listeria monocytogenes*
Disease
- Listeriosis
Diagnosis
- Can be difficult – symptoms up to 30 days after ingestion
- ‘At risk’
  - Pregnant women
  - Infants
  - Elderly / hospitalised persons

Human listeriosis in England and Wales

Data from HPA, http://www.hpa.org.uk/Topics/InfectiousDiseases/InfectionsAZ/ listerialistiosis
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Age specific rates of listeriosis in England and Wales 1990-2006  HPA unpublished data

Estimated that >500 deaths occur due to food poisoning
listeriosis is now the most common cause of death

FSA Annual Report of the Chief Scientist 2006/7

Increase almost exclusive to patients over 60 years of age with bacteraemia

EU reported listeriosis cases

Data from: The Community Summary Report on Trends and Sources of Zoonoses, Zoonotic Agents, Antimicrobial Resistance and Foodborne Outbreaks in the European Union - The EFSA Journal
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**EU reported listeriosis cases**

Significant increase in Denmark, Finland, France, Germany, The Netherlands, Spain and UK (52% of total population of the EU).

**FOOD ASSOCIATED WITH LISTERIOSIS**

**DAIRY PRODUCTS**
- Soft cheese
- Milk
- Ice cream/soft cream
- Butter

**MEAT PRODUCTS**
- Cooked chicken
- Turkey frankfurters
- Sausages
- Pâté and rillettes
- Pork tongue in aspic
- Meat pies

**VEGETABLE PRODUCTS**
- Coleslaw salad
- Vegetable rennet
- Salted mushrooms
- Alfalfa tables
- Raw vegetables
- Pickled olives
- Rice salad
- Cut fruit
- Sandwiches

**FISH PRODUCTS**
- Fish
- Shellfish
- Shrimps
- Smoked fish, shellfish
- Cod roe

**Listeria monocytogenes and melon**

USA Multistate distribution
- Four PFGE types
- 146 cases (July–Sept 2011)
  - Age <1 to 96
  - Median 77 years
  - 142 hospitalized
  - 30 deaths
  - All types recovered from:
    - Single farm (Colorado)
    - Patients homes
    - Whole cantaloupe melon
    - Cut cantaloupe melon
  - Equipment and environment at packing facility

September 2011, FDA press statement for voluntary recall of Rocky Ford-brand cantaloupes

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**Listeriosis outbreak, epidemic curve**

Listeria and melon, a problem in the UK?

LACORS/HPA Food survey 2002
- 997 samples of pre-cut fruit
- L. monocytogenes present in 86
  - 1 at 20-100/g
  - 1 at 260/g

Local HPA Survey of cut fruit in the North West 2011-12
- ~680 samples
- L. monocytogenes present in 6% (2 at 10-100cfu/g)
- S. Newport present in 1
- Unpublished, on going study

UK outbreak associated with pork pies. 2010-12

**Index case**
- Meningitis SE England July 2012 due to L. monocytogenes AFLP type 4.I.74
- Patient linked illness with pork pie consumption in East Midlands
- Family arranged for purchase and transport of a pork pie
- Pork pie was contaminated with L. monocytogenes AFLP type 4.I.79

**Retrospective analysis**
- Additional 13 Listeriosis cases (2010-12) due to indistinguishable or very similar types (AFLP types i.e. 4.I.74 and 4.I.79)
- All cases occurred in South Yorkshire or the East Midlands
- Food consumption history from nine patients, reported consuming pork pies
- Cluster analyses indicated that the two L. monocytogenes types were significantly associated with pork pie consumption (OR: 16.4 (3.20 – 113.2), p<0.0001).
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**Listeriosis, Canada 2008**

- 57 cases
- 22 deaths
- Outbreak associated with consumption of cold meats and prepared sandwiches produced by Maple leaf Foods
- Canada’s biggest food processor
- Withdrawal of 220 products
- Contamination from factory sites, possibly slicing machines
- Shares lost 25% of their value in August-September 2008

[Link](http://www.listeriosis-listeriose.investigation-enquete.gc.ca/lirs_rpt_e.pdf)

**Foodborne listeriosis associated with pre-prepared sandwiches in hospitals England and Wales and Northern Ireland 1999-2011**

<table>
<thead>
<tr>
<th>Year</th>
<th>Regions</th>
<th>Cases (deaths)</th>
<th>Cross-contamination and or inadequate cleaning at factory</th>
<th>Inappropriate temperature and/or shelf life control at hospital</th>
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<tbody>
<tr>
<td>1999</td>
<td>NE England</td>
<td>4 (1)</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>2003</td>
<td>S Wales</td>
<td>2 (0)</td>
<td>Yes</td>
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<tr>
<td>2003</td>
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<td>2004</td>
<td>SE England</td>
<td>2 (1)</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>2008</td>
<td>North West</td>
<td>3 (2)</td>
<td>Yes</td>
<td>Yes</td>
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<tr>
<td>2008</td>
<td>N.Ireland</td>
<td>1 (0)</td>
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<td></td>
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<tr>
<td>2010</td>
<td>North East</td>
<td>3 (1)</td>
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<td>Yes</td>
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<tr>
<td>2011</td>
<td>West Midlands</td>
<td>3 (0)</td>
<td>Yes</td>
<td></td>
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<tr>
<td>2012*</td>
<td>N.Ireland</td>
<td>6 (1)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Little et al., Hospital acquired listeriosis associated with sandwiches in the UK: a case for concern. J Hosp Infect 2012 and * unpublished N.Ireland data.

**Sandwiches in Hospitals**

Sandwich industry is worth over £3.5 billion, employs approx 300,000 people and >3,000,000,000 sandwiches purchased each year in UK

Eight outbreaks, common contributing factors do not explain the increase

Increase in pre-prepared sandwiches in hospitals

The current (contracted) annual value of sandwich sales into the NHS is £13m approx

16 million sandwiches at an average price of £0.80

This does not account for the sandwiches that are bought from non-contracted suppliers or made in-house.

Data from NHS Supply Chain
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**L. monocytogenes** contamination of pre-prepared sandwiches. LACORS/HPA Food study 2009

<table>
<thead>
<tr>
<th>Setting</th>
<th>Total</th>
<th>No (%) contaminated</th>
<th>&gt;10²/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hospital and institutional</td>
<td>878</td>
<td>14 (2%)</td>
<td>0</td>
</tr>
<tr>
<td>Retail</td>
<td>1392</td>
<td>61 (4%)</td>
<td>3 (0.2%)</td>
</tr>
</tbody>
</table>

**Salmonellosis**

Two Salmonella species (more than 2500 different antigenic types):

- *Salmonella enterica*
  - I. enterica
  - II. arizonae
  - III. Arizona
  - IV. diarizonia
  - V. houtanense
  - VI. indica

- *Salmonella bongori* (previously sub-species V)

Widely distributed

- Zoonoses
- Most serotypes that infect mammals are found in subspecies I

**Salmonella – Traditional food animal reservoirs and food vehicles**

Food animals

Foods
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Where else do we find Salmonella?

Imported “exotic foods”

- SEA FOOD
- COCONUT
- CURRY
- SPICES
- FROGS’ LEGS
- PET FOODS
- U.K.

90% of reptiles carry Salmonella

Salmonella spread via faeces & urine
Infection by claw scratches & bites (!)

S. Typhimurium DT191A, tetr, 2008:

Case locations

Epi investigations

8/10 families kept reptiles

Mostly a snake
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‘Pinkies’!

Thanks to:
Katy Hawker, Chris Lane
For slides and information
Hawker et al. Epidemiol Infect 2011;139:1254-61

S. Typhimurium DT 191A-infection pathways

Salmonella
→ Mouse
→ Snake/reptile
→ Reptile handler
→ Environment
→ Baby/child

Tetracycline-resistant Salmonella in reptiles:– control measures

Local

International

Thanks to Chris Lane and Katy Hawker for information
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**Botulism**

Rare but potentially fatal disease caused by neurotoxins of *Clostridium botulinum*

Various forms:
- Foodborne botulism
- Wound botulism
- Infant botulism

**Human Botulism**

*Foodborne botulism,*

intoxication by ingestion of preformed toxin in food where the organism has grown

**Clostridium botulinum**

- Gram-positive anaerobic rod
- Heat-resistant spores
- Naturally present in soil, dust, marine sediments, food
- Six antigenically distinct neurotoxins (BoNTs) A to G
- Types A, B, E and F associated with human disease,
- C and D animal disease, G no disease
- Four distinct groups of organisms by phenotypic characteristics and DNA homology – human disease groups I, II
- BoNT can also be produced by rare strains of *C. butyricum* and *C. baratii*
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**Botulism**

**Onset**

- **Foodborne**: 12-36 hrs can be 6 hrs - 8 days
- **Infant**: hours to over a week - floppy baby syndrome
- **Wound**: variable 2-3 days to 18 days

**Recovery** in 2-8 weeks requires regeneration of new neuromuscular connections - resprouting of nerve terminals

**Prolonged ventilatory support, some for >7 months**

**Severity** depends on toxin type, dose, individual susceptibility

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**PCR detection of BoNT genes**

(Taqman-based real time assay)

**Rapid detection method**

Clinical specimens put into CMB, sent to ref lab and PCR assay results within <3 hours of receipt

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**Infant botulism in the UK and Ireland 2001-2011**

<table>
<thead>
<tr>
<th>Year</th>
<th>Age</th>
<th>Sex</th>
<th>BoNT</th>
<th>Laboratory diagnosis using Faeces/rectal wash out</th>
<th>Risk factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>8 months</td>
<td>M</td>
<td>A</td>
<td>PCR detection of organism and 'Toxin'</td>
<td>Weaning</td>
</tr>
<tr>
<td>2007</td>
<td>4 months</td>
<td>M</td>
<td>B</td>
<td>PCR detection of organism and 'Toxin'</td>
<td>Antibiotic treatment</td>
</tr>
<tr>
<td>2009</td>
<td>2 months</td>
<td>F</td>
<td>A</td>
<td>PCR detection of organism</td>
<td>Food honey</td>
</tr>
<tr>
<td>2009</td>
<td>2 months</td>
<td>M</td>
<td>A</td>
<td>PCR detection of organism</td>
<td>Food honey</td>
</tr>
<tr>
<td>2010</td>
<td>3½ months</td>
<td>M</td>
<td>E</td>
<td>PCR detection of organism</td>
<td>Fed honey, terrapins as pets</td>
</tr>
<tr>
<td>Jan 2011</td>
<td>2 weeks</td>
<td>M</td>
<td>E</td>
<td>PCR detection of organism</td>
<td>Terrapins as pets</td>
</tr>
<tr>
<td>April 2011</td>
<td>3½ months</td>
<td>F</td>
<td>A</td>
<td>PCR detection of organism</td>
<td>Fed honey</td>
</tr>
<tr>
<td>June 2011</td>
<td>3½ months</td>
<td>M</td>
<td>A</td>
<td>PCR detection of organism</td>
<td>Fed honey</td>
</tr>
<tr>
<td>Jun 2011</td>
<td>4 months</td>
<td>M</td>
<td>B</td>
<td>PCR detection of organism</td>
<td>Breasts and bottle fed, home being renovated - dusty conditions</td>
</tr>
</tbody>
</table>
Infant Botulism

1978-2007 infant botulism very rare in the UK – 6 cases total
Since 2007 there have been 8 cases, 3 occurring in past year
Most cases the source of spores never identified – spores swallowed from the environment, honey a known risk factor, formula milk, weaning and antibiotic treatment alter gut flora providing opportunity for spores to germinate
5/8 recent cases with history of honey consumption
Increase in cases due to increased recognition?
Still delay in diagnosis – IB not thought of and infant initially treated for sepsis

2 cases of type E infant botulism
UK and Ireland, 2010
Both cases were discharged as well and readmitted to hospital
Illness of short duration compared with IB due to other BoNT types
Families kept yellow bellied turtles as pets:
- microbiological evidence that strain from pet turtle tank same subtype as isolate from Irish infant
- ascertained retrospectively that family of UK case also kept turtles as pets

Prevention of infant botulism:
- Do not feed honey to infants
- Do not keep reptiles as pets
Foodborne botulism in the UK and Ireland, 1998-2011

<table>
<thead>
<tr>
<th>Year</th>
<th>Cases</th>
<th>Food</th>
<th>Preparation and origin</th>
<th>Toxin Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>1998</td>
<td>2</td>
<td>Bottled mushrooms</td>
<td>Home made in Italy</td>
<td>B</td>
</tr>
<tr>
<td>2003</td>
<td>1</td>
<td>Sausage</td>
<td>Home made in Poland</td>
<td>B</td>
</tr>
<tr>
<td>2004</td>
<td>1</td>
<td>Hummus</td>
<td>Commercial, UK</td>
<td>Not confirmed</td>
</tr>
<tr>
<td>2005</td>
<td>1</td>
<td>Not known</td>
<td>Returned from Georgia</td>
<td>A</td>
</tr>
<tr>
<td>2005</td>
<td>1</td>
<td>Preserved pork</td>
<td>Home made in Poland</td>
<td>B</td>
</tr>
<tr>
<td>2006</td>
<td>1</td>
<td>Preserved pork</td>
<td>Home made in Poland</td>
<td>A/B</td>
</tr>
<tr>
<td>2008</td>
<td>1</td>
<td>Polish sausages</td>
<td>Polish national in Ireland</td>
<td>B</td>
</tr>
<tr>
<td>2010</td>
<td>1</td>
<td>Not known</td>
<td>Returned from Algeria</td>
<td>B</td>
</tr>
<tr>
<td>2011</td>
<td>3</td>
<td>Korma sauce</td>
<td>Commercially prepared, UK</td>
<td>A</td>
</tr>
</tbody>
</table>

Recent incident of foodborne botulism in Scotland

- Food history: foods eaten predominantly by ill children only
- 2/3 siblings Scotland in PICU, ventilated, and treated with antitoxin
- Tested 5 possible foods

- BoNT detected in patient serum by MBA
- C. botulinum type A detected by PCR in rectal washouts
- BoNT A detected in remnants of Korma sauce
- C. botulinum type A detected by PCR in Korma sauce

This led to national recall of Korma sauce product

Botulism in Eastern Europe

- Historically higher rates: Georgia highest rate worldwide
- Botulism more common where: home or small scale preservation of food practiced
- Economic decline leads to:
  - lack of energy for cooking food
  - lack of clean water and food preservatives
  - food shortages
- Migrant workers and immigrants:
  - >200,000 Poles moved to UK since EU succession
  - Food may be brought back from other member states for consumption
Threlfall – We never look for it because we never find it; emerging food-borne pathogens you need to know about

Verotoxigenic *Escherichia coli* (VTEC)

**Disease/ Symptoms**
- Haemolytic Uraemic Syndrome (HUS)
- Haemorrhagic Colitis (HC)
- Diarrhoea
- (Asymptomatic)

**Vulnerable population**
- Infants and young children
- Adults

**Serotypes**
- O157:H7
- Non-O157 e.g. O26:H1, O103:H2, O121:H19, etc
- [O104:H4]

Verotoxigenic *Escherichia coli* (2)

Verocytotoxin-producing *E. coli*

Traditionally:
- Produce verocytotoxin (Shiga toxin).
- Possess genes coding for VT production – *vtx1*, *vtx1* and *vtx2* (or variants).
- Possess intimin-encoding *eae* gene (necessary for attachment and effacement).

Thus: *vtx* positive, *eae* positive

Common feature of O157:H7, O26:H1, O103:H2, O121:H19 etc.

Verotoxigenic *Escherichia coli* (3)

2011 *E. coli* O104:H4 outbreak

- 4231 confirmed VTEC cases, with 852 HUS cases and 54 deaths.
- Vehicle of infection: Sprouted imported fenugreek seeds.

Strain:
- Produced verocytotoxin (Shiga toxin).
- Possessed gene coding for VT production – *vtx2*, (*stx2*).
- Did NOT possess *eae* gene.

Thus: *vtx/stx*-positive, *eae*-negative.

Strain also possessed other virulence characteristics common to enteroaggregative *E. coli* (EAEC)
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**Verotoxigenic Escherichia coli (4)**

**2011 E. coli O104:H4 outbreak**
- Strain vtx-positive, eae-negative
- New molecular paradigm for identification of such strains in RTE foods therefore required.
- Because of EAEC strain background, screening for virulence-associated, chromosomally-encoded aaiC (secreted protein of EAEC) and plasmid-encoded aggR (regulator) genes, as well as for vtx and eae genes, now recommended.


**Hepatitis E (HEV) (1)**

- Non-enveloped spherical RNA virus classified as a Hepevirus.
- First recognised as a distinct human disease in the 1980s.
- Transmitted by the faecal-oral route.
- Common in Asia, Africa and Central America, particularly where sanitation is poor.

**Hepatitis E (HEV) (2)**

**Disease**
- Usually produces mild disease but in rare cases can prove fatal, particularly in pregnant women.
- Relatively uncommon cause of viral hepatitis in the UK.
- Increasingly frequent reports of cases from industrialised countries involving patients who have not travelled abroad.
- HEV figures may be underestimates since many laboratories do not routinely test for the virus.
Threlfall - We never look for it because we never find it; emerging food-borne pathogens you need to know about

**Hepatitis E (HEV) (3)**

**Surveillance (HPA UK figures)**

<table>
<thead>
<tr>
<th>Year</th>
<th>2003</th>
<th>2004</th>
<th>2005</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>*2012</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. confirmed cases</td>
<td>124</td>
<td>149</td>
<td>329</td>
<td>289</td>
<td>162</td>
<td>176</td>
<td>175</td>
<td>274</td>
<td>456</td>
<td>566</td>
</tr>
</tbody>
</table>

*provisional number

**Food-borne infections**

- Shellfish implicated as potential source of infection in a recent outbreak among UK passengers on a cruise ship.
- Study by HPA and the Veterinary Laboratories Agency found that patients with non-travel associated HEV were infected by the HEV genotype 3 strain carried by British pigs.
- Therefore possible that pigs might be a source of infection in people that acquire their infection in the UK.
- Could also be a source of infection common to both pigs and humans.

**Hepatitis E (HEV) (4)**

‘Emerging pathogens we need to know about’

‘There are known knowns; there are things we know we know’.
- *Listeria, Salmonella, C. botulinum, VTEC, HEV,* all cause human disease, which can be very severe.

‘We also know there are known unknowns’
- under-diagnosis and under-ascertainment.
- food vehicles and food animal reservoirs.
- Infallible methods of diagnosis?
  - of detection in foods.

‘But there are also unknown unknowns – the ones we don’t know we don’t know !!’
Threlfall - We never look for it because we never find it; emerging food-borne pathogens you need to know about

Thank you for listening

And particular thanks to:

Jim McLauchlin and Kathie Grant (HPA, UK)

For slides and information
Peixe - Biting off more than we can chew? How multiple drug resistance is affecting how we manage food-borne infection

Emergence and spread of resistant bacteria

Antibiotic use in food-producing animals
Peixe - Biting off more than we can chew? How multiple drug resistance is affecting how we manage food-borne infection
Peixe - Biting off more than we can chew? How multiple drug resistance is affecting how we manage food-borne infection
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Salmonella Kentucky

- Carbapenems - not hydrolysed by most β-lactamases, including AmpC β-lactamases and extended-spectrum β-lactamases (ESBLs)

Salmonella non-typhoid. Carbapenemase producers

Escherichia coli. Clinical relevance

Antimicrobials are critical for treating EsFC infections but not for diabetogenic E. coli
Peixe - Biting off more than we can chew? How multiple drug resistance is affecting how we manage food-borne infection
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Campylobacter infections

- 27% broiler meat (3.1% - 90.0%)
- 0.6% pig meat
- 0.4% bovine meat

EU countries (2010)

- 27 - 71%
- 0 - 8.6%
- 6.4 - 51.6%

NETHMAP, 2012

Humans Netherland

Campylobacter and Resistance

Conclusions
Peixe - Biting off more than we can chew? How multiple drug resistance is affecting how we manage food-borne infection
Inglis - Jettison your gels! New methods for typing food-borne pathogens

Out with the old?


Inglis - Jettison your gels! New methods for typing food-borne pathogens

**Sequencing**


>> sequencing
Multilocus sequence typing of Salmonella strains by high-throughput sequencing of selectively amplified target genes.


Chips with everything?


Arrays


Inglis - Jettison your gels! New methods for typing food-borne pathogens

MALDI-TOF


Integration

- Clinico-pathological features
- Epidemiology
- Cell & molecular biology
  - Omics
  - Functional dynamics

Bioinformatic load

- Internal data generation stream
  - Wet molecular biology
  - Dry computational biology
  - Processing power
- External/historic data
  - Concordance with previous typing methods
  - Population structure
- Parallel analysis
Inglis - Jettison your gels! New methods for typing food-borne pathogens

Does it do the job better?

<table>
<thead>
<tr>
<th>Method</th>
<th>PFGE</th>
<th>MLST</th>
<th>MLVA</th>
<th>NGS</th>
<th>MMS-SNP</th>
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<tbody>
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<td>discrimination</td>
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Head-to-head

1. Level of discrimination
2. Consistency
3. Technical complexity
4. Speed of operation
5. Availability
6. Cost

Notifiable food-borne infections

- *Salmonella*
- *Listeria*
- *E.coli*
- *Campylobacter*
- *Clostridium*

- Consensus methods
- Multiple methods
- Data transfer & sharing
Inglis - Jettison your gels! New methods for typing food-borne pathogens

Emerging infections


So, are we done with gels?

• Still using PFGE
• Options for PW:
  – MLVA
  – MMS/SNP
  – Targeted sub-WGS

Thanks to

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