



Toolbox development for microbial source tracking in water sources and catchments

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Microbial source tracking (MST)

- A relatively new and developing approach that aims to identify the origins of faecal pollution in water
- Human faecal pollution considered greater risk
- Targeted, timely and cost effective management
- *Premise*: enteric microbes exhibit host-specific characteristics due to host's gastrointestinal tract and differences in dietary compositions
- No 'gold standard' method or target

Microbial source tracking

Point sources

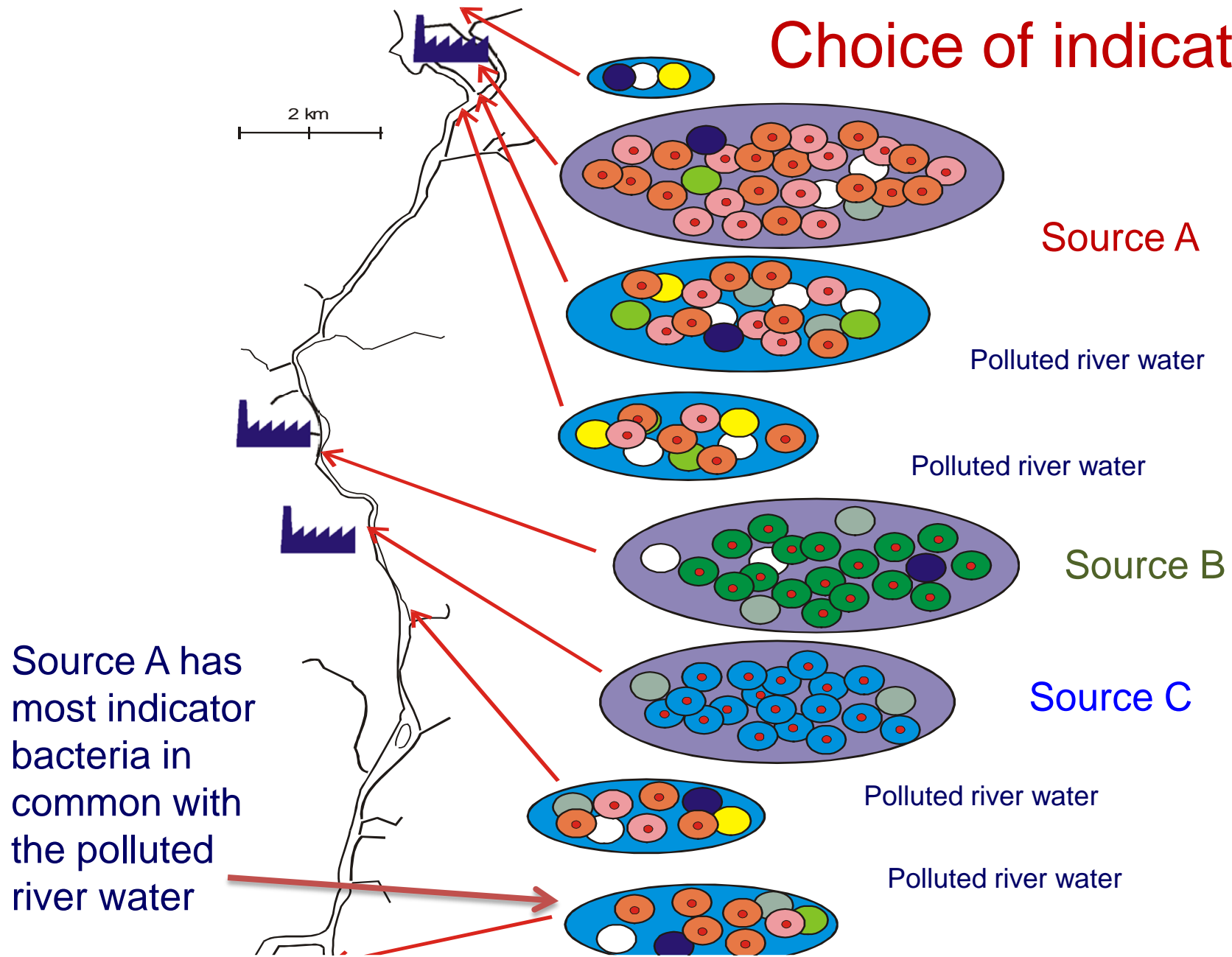
- Defined points of influx
- WWTP
- Industrial waste
- Combined sewage overflow

Non-point sources

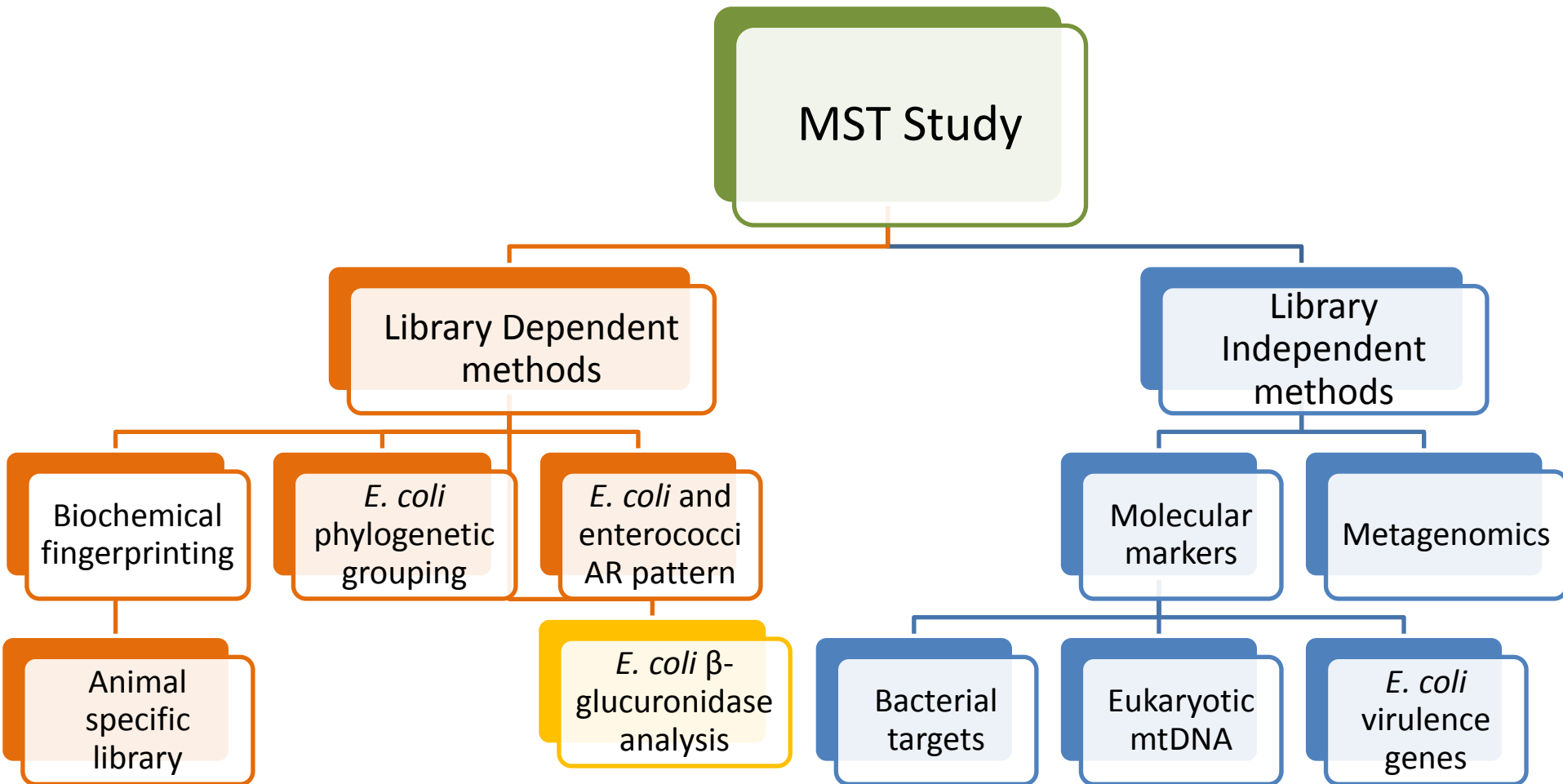
- Surface runoff
- Agricultural overflow
- Septic systems
- Groundwater discharge
- Seepage
- Direct/recreational contact
- Aquaculture practices



Choice of indicator



Toolbox development for microbial source tracking in water sources and catchments



Evaluation of each method has to be done (in a DB study)

Collection and analysis of faecal materials from humans/animals for double blind study (problems!)

Requirements:

1. Pool samples (ideally from as many different farms/animals as possible)
2. Fresh faecal samples (simultaneous collection of faeces from animals, transport to laboratory, testing etc.)
3. Reproducibility of results and retesting of faecal samples (inter and intra assay variation)
4. Stability of bacteria in faeces

Faecal preservation study

Investigation of *E. coli* and enterococci:

1. Changes in number of strains in faeces
 2. Survival of different types (clones)
 3. Changes in population diversity
- after preserving faeces at -20°C and -80°C for **5** weeks

Animal species tested

1- Horse ; 2- pig ; 3- cow ; 4- chicken ; 5- dog
6- bird and 7-humans

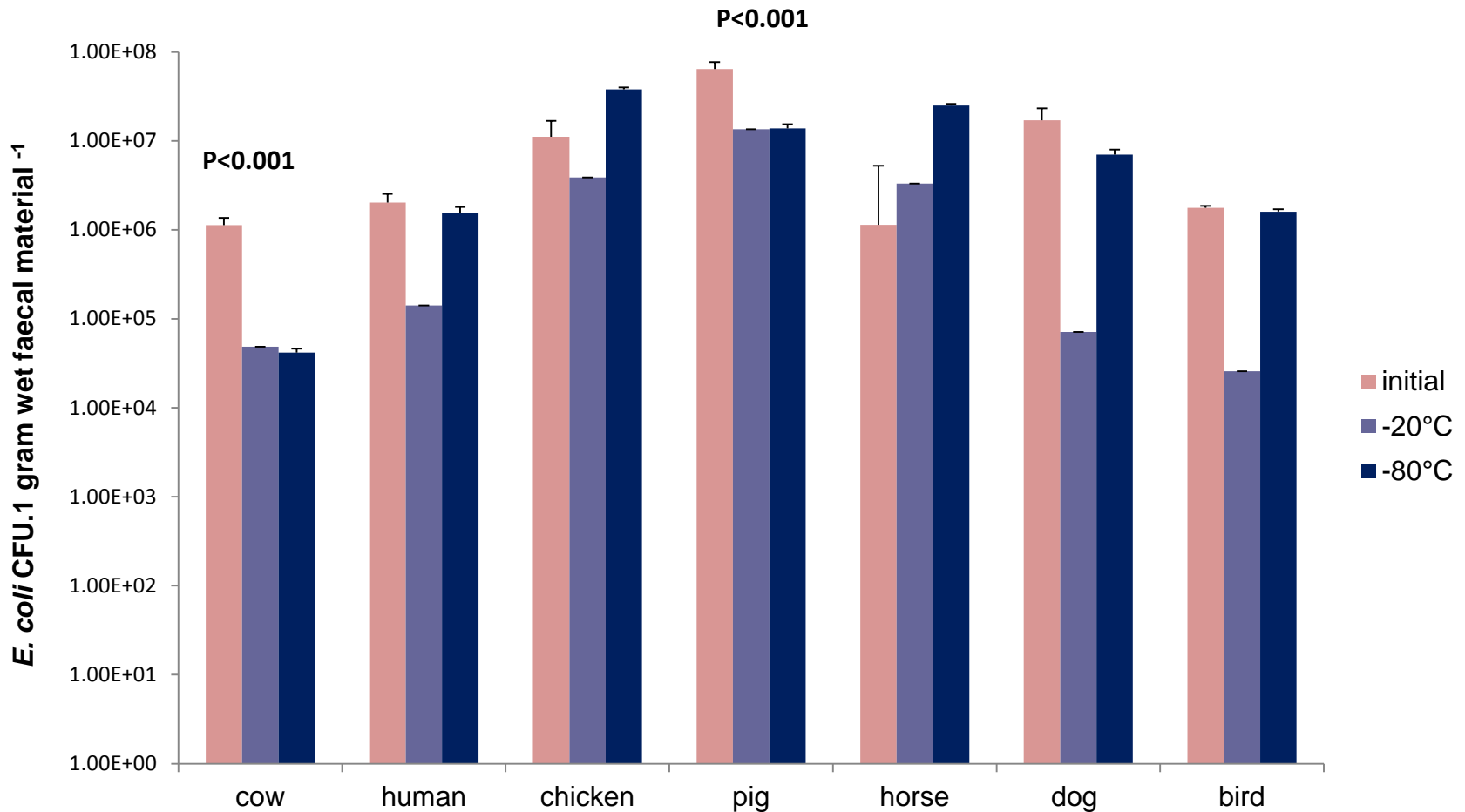
Up to 10 (where possible) faecal samples from each species were collected, pooled and divided.

A: tested immediately

B: preserved at -20°C

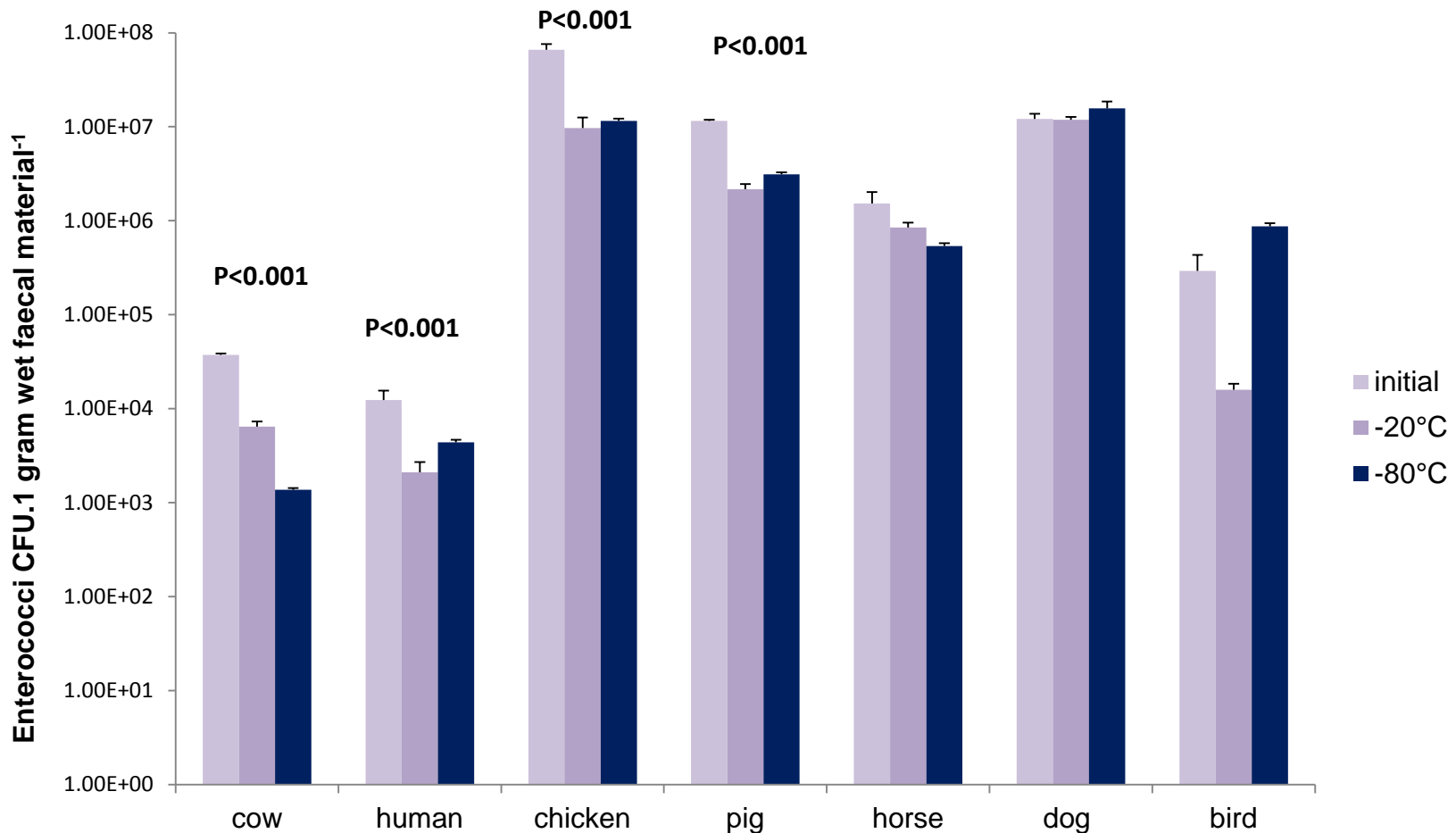
C: Preserved at -80°C

Faecal preservation study (*E. coli*)



Changes in the number of *E. coli* in faecal samples of animal species tested after preservation in -20°C and 80°C.

Faecal preservation study (enterococci)



Changes in the number of enterococci in faecal samples of animal species tested after preservation in -20°C and 80°C.

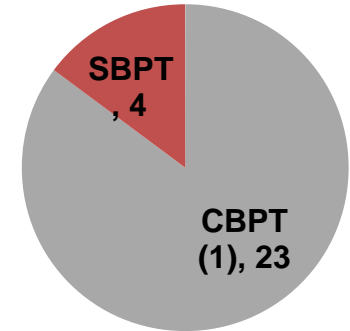
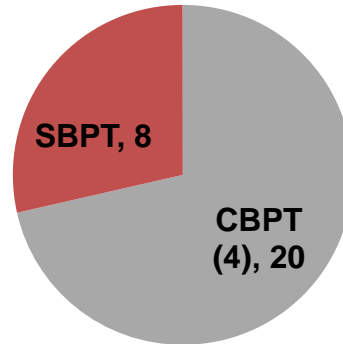
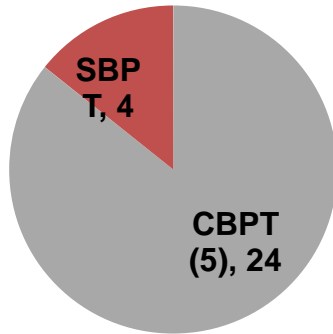
Cow

Initial

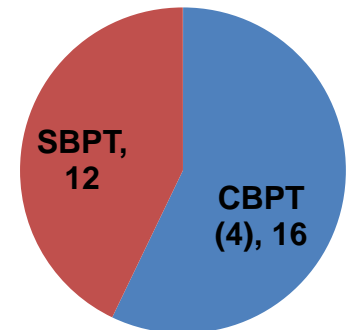
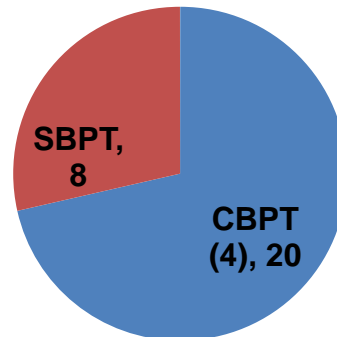
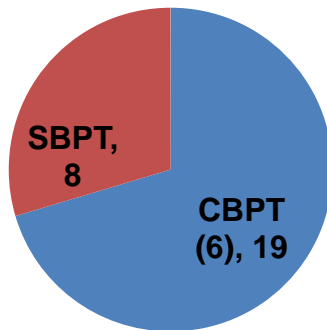
After 5 weeks
-20°C

After 5 weeks
-80°C

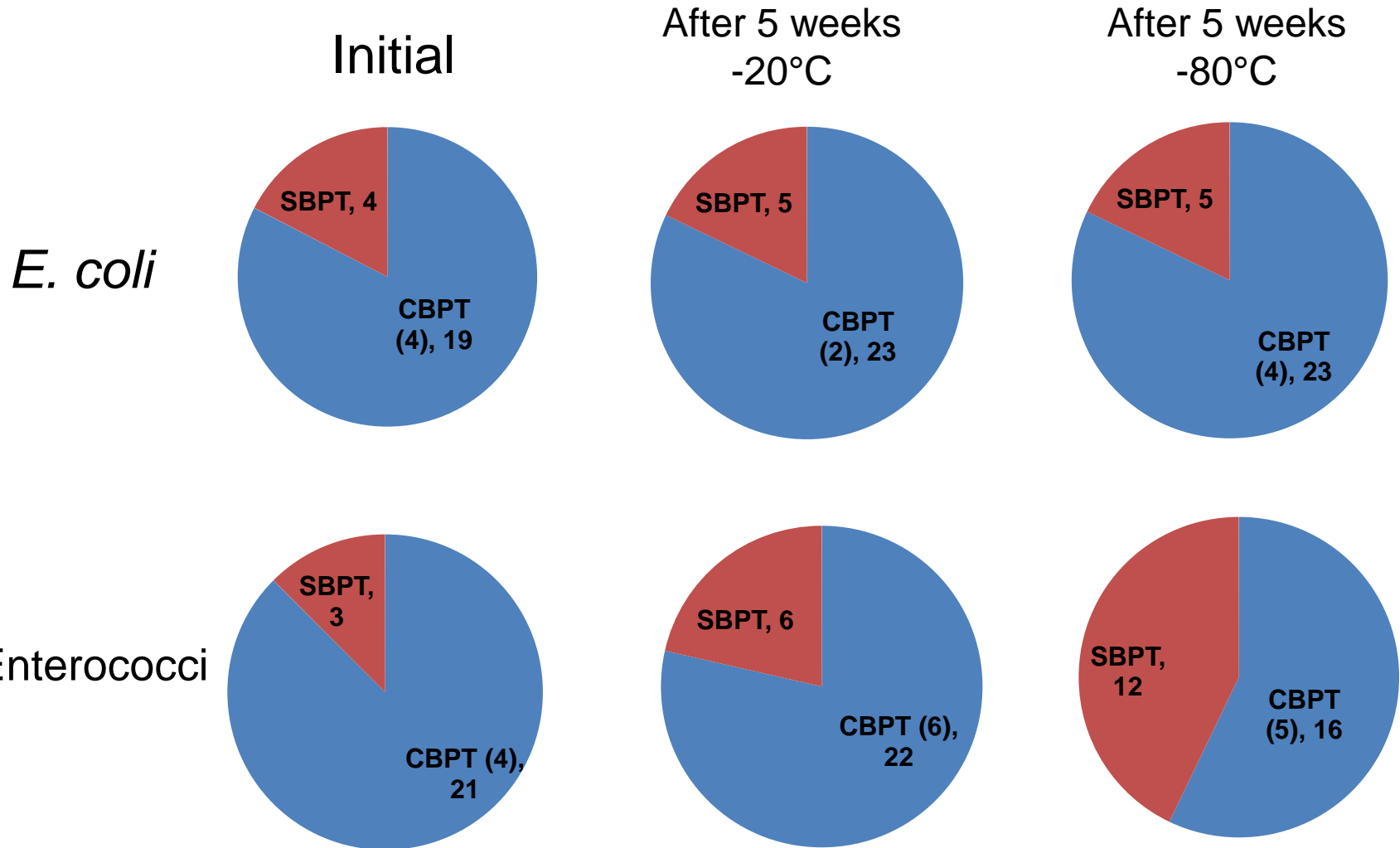
E. coli



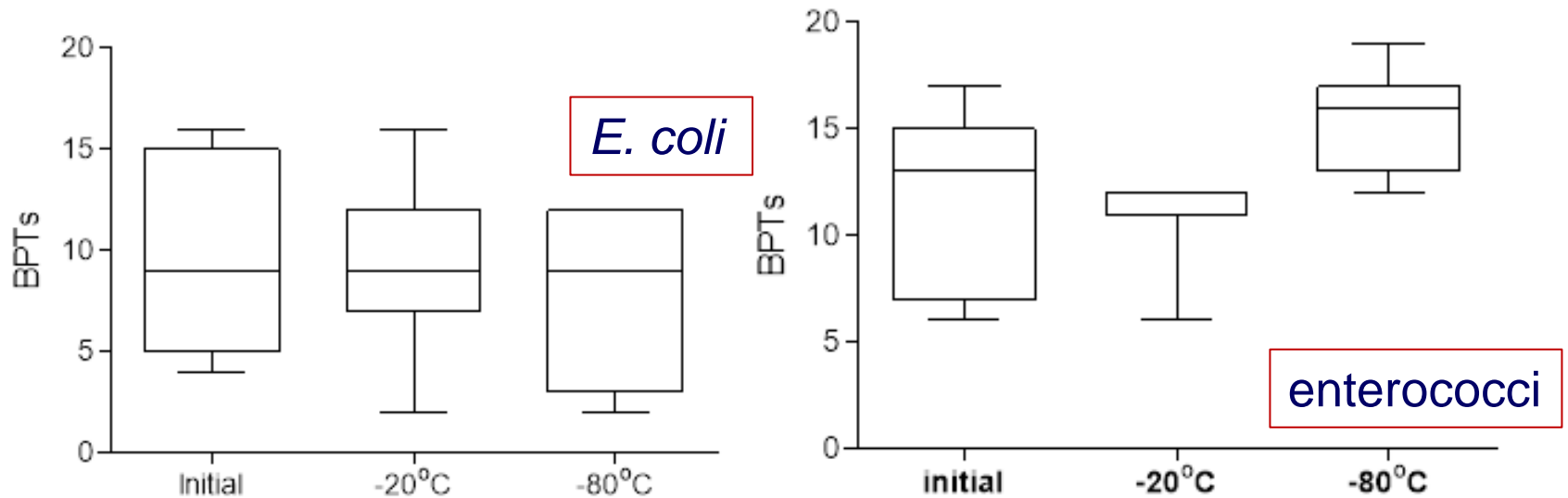
Enterococci



Human



Stability of common clones



The population structure of *E. coli* and enterococci strains was relatively stable irrespective of changes in their concentration

Preservation at -80°C of faecal sample was superior than -20°C and this was species-dependent

Double blind study

Species /host specific MST markers used

Bacteroides: GenBac

Human: BacHum, HF183,

Cow: CowM3, CF128, BacC305R

Ruminant: RumB1F, BacPreR, Bac32F, RumD2

Horse: HoF597F, Bac708R

Dog: BacCan545, BacUNI69

Clostridium: Chicken: CP349

Helicobacter: Chicken/Avian: GFDF, GFDR

Cattellicoccus: Chicken/Avian: GFDF, GFDR

E. coli: Dog: *papG* allele III; Swine: *stx2* (PSVII)

Eukaryotic: DmtDNA, Swine: SmtDNA

Methanogen: Swine: P23-2, P232R

Enterococci: *esp* gene

Source-specific library (Biochemical fingerprinting): *E. coli* and *enterococci*

Antibiotic resistance profile: *E. coli*, *enterococci*

Phylogenetic grouping

Beta-glucuronidase gene as a novel MST marker: *E. coli*

β -glucuronidase gene in *E. coli*



- β -glucuronidase gene (*uidA/gusA/gurA*)
 - 1812 bp: catalyses wide variety of β -glucuronides
 - ~ 95% of all naturally occurring *E. coli*
 - Variation within gene (specific to a host)
- The primer set: 52F and 1020R (Stratton et al., 2010)
 - 518 bp fragment
 - 59 points of variation amongst animal/humans

10/59 points of variation were selected

Position on 518 bp β -glucuronidase fragment	Animal base nucleotide*	Human base nucleotide
59	G	A,T,C
95	C	T
185	C	T
203	T	C
245	C	T*
302	G	A
322	T	G (few T)*
326	C	A*
392	T	C*
410	G	A*

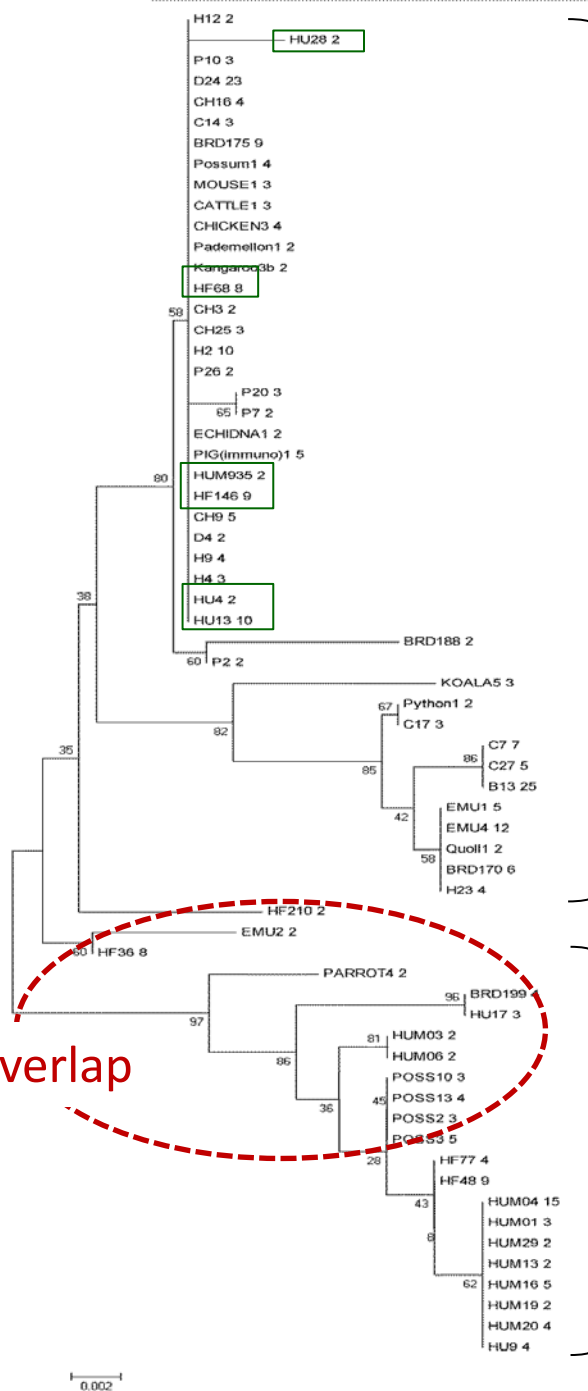
* indicates a general trend with minor differences

E. coli collection tested (n=318)

Source of isolates		No. of C- types	No. of isolates
Human	Faecal	16	89
	Urine	5	23
Animal (domestic)	Cow	5	21
	Pig	6	17
	Chicken	5	18
	Dog	2	25
	Horse	5	23
Animal (wild fauna)	Birds (mixed)	5	46
	Kangaroo	1	2
	Possum	5	19
	Echidna	1	2
	Koala	1	3
	Pademelon	1	2
	Emu	3	19
	Quoll	1	2
	Parrot	1	2
	Python	1	2
	Mouse	1	3

β -glucuronidase gene in *E. coli*: fragment analysis

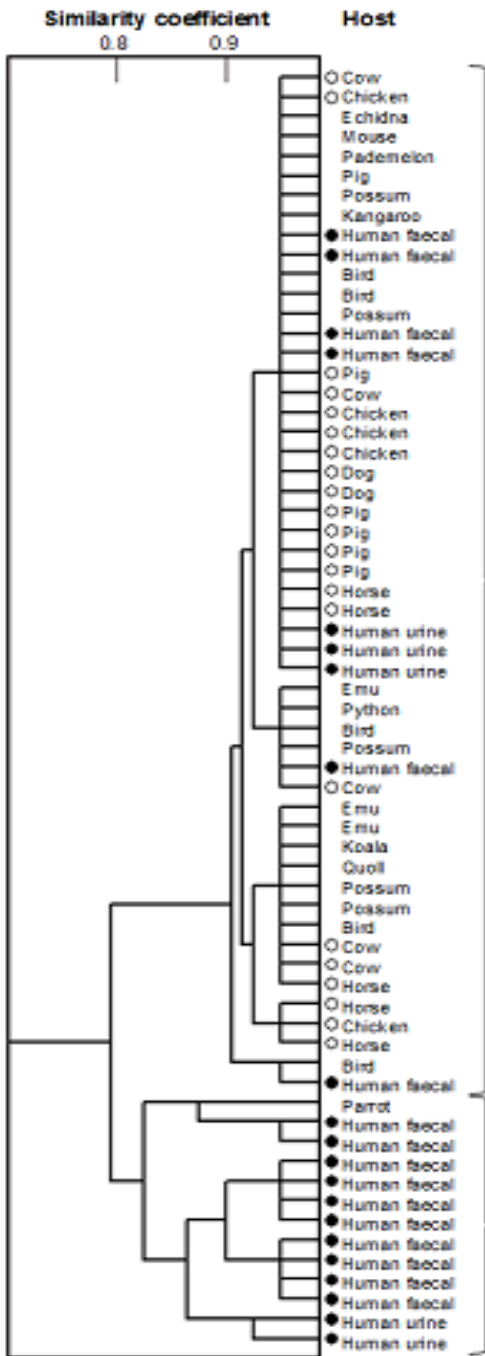
Animal clade



Human clade

Neighbour joining cluster analysis
(MEGA software)

β -glucuronidase gene in *E. coli*: fragment analysis



Animal cluster

Human cluster

Source of isolates	Human clade	Animal clade	no. of isolates
Neighbour joining cluster analysis			
Human (faecal)	82%	18%	89
Human (urinary)	56.5%	43.5%	23
Animal	8.7%	91.2%	206
Numerical analysis and UPGMA clustering			
Human (faecal)	84.3%	15.7%	89
Human (urinary)	73.9%	26.1%	23
Animal	1.0%	99.0%	206

Numerical analysis using UPGMA clustering

DB testing individual faecal samples

Correct identification
false -ve should have been present but wasn't
false +ve present but not supposed to be

	General	Human		Cow			Chicken	Avian (chicken)		Ruminant		Horse	Dog			Swine			
	GenBac	BacHum	HF183	COWM3	CF128 Bac708R	CF128 BAC305R	CP349	GFDF GFDR	GFCF GFCR	RumB1F BacPreR	Bac32F RumD2	HoF597F BAC708R	BacCan545 BacUNI690	papG Allele III F and R	DmtDNA F DmtDNA R	P23-2 P23-2R	SmtDNA F SmtDNA R	stx2 (PSVII)	
	Bacteroides	Bacteroides	Bacteroides	Bacteroides	Bacteroides	Bacteroides	Clostridium	Helicobacter	Catellicoccus	Bacteroides	Bacteroides	Bacteroides	Bacteroides	E. coli	eukaryotic	Methanogen	eukaryotic	E. coli	
Human	+		+				+			+							+	(faint)	+
Pig	+				+	+				+						+	+	+	+
Cow	+			+	+	+				+	+								+
Horse	+					+						+							+
Dog	+		+						+				+		+				+
Bird	+								+										+
Chicken	+						+	+										+	+

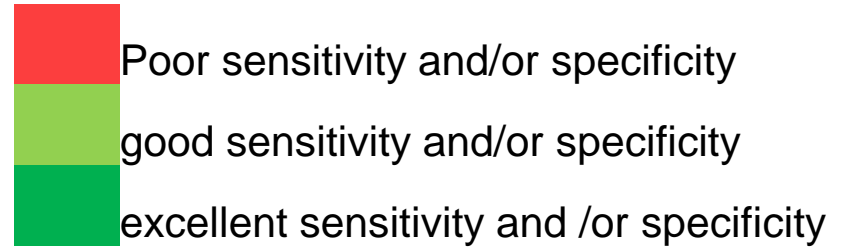
DB testing mixed (up to 7) faecal samples

No. of mixed faecal samples

Correct identification
 false -ve should have been present but wasn't
 false +ve present but not supposed to be

6	Dog	Human	Cow	Horse	Bird	Chicken	Pig
3	Dog	Human	Cow		Bird	Chicken	
4	Dog		Cow	Horse	Bird	Chicken	
6	Dog	Human	Cow	Horse	Bird	Chicken	Pig
5	Dog	Human	Cow	Horse	Bird		Pig
2			Cow	Horse			
6	Dog	Human	Cow	Horse	Bird		Pig
3	Dog	Human	Cow		Bird	Chicken	Pig
4	Dog	Human	Cow	Horse	Bird	Chicken	Pig
5	Dog	Human	Cow		Bird		Pig
7	Dog	Human	Cow	Horse	Bird	Chicken	Pig
4	Dog	Human	Cow		Bird	Chicken	Pig
4	Dog	Human	Cow		Bird	Chicken	Pig
3	Dog		Cow	Horse	Bird	Chicken	

Sensitivity and Specificity of the molecular markers



	Human		Cow			Chicken	Avian (chicken)		Ruminant		Horse	Dog			Swine	
	BacHum	HF183	COWM3	CF128 Bac708R	CF128 BAC305R	CP349	GFDF GFDR	GFCF GFCR	RumB1F BacPreR	Bac32F RumD2	HoF597F BAC708R	BacCan545 BacUNI690	papG Allele III F and R	DmtDNA F DmtDNA R	P23-2 P23-2R	SmtDNA F SmtDNA R
	Bacteroides	Bacteroides	Bacteroides	Bacteroides	Bacteroides	Clostridium	Helicobacter	Catelllicoccus	Bacteroides	Bacteroides	Bacteroides	Bacteroides	E. coli	eukaryotic	Methanogen	eukaryotic
Sensitivity	70%	50%	80%	100%	70%	70%	80%	90%	70%	80%	90%	100%	10%	100%	70%	100%
Specificity	73%	64%	82%	46%	46%	80%	80%	46%	54%	100%	100%	64%	91%	100%	91%	80%

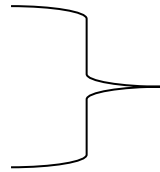
Research direction

To improve and add to:

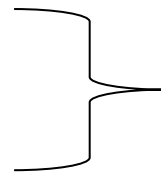
- the current range of proven markers particularly eukaryotic markers, viral DNA/RNA and the various bacterial strains
- Apply the markers and MST library independent approaches to multiple catchments, water-ways
- Work towards a rapid and possibly infield array assay with a turnaround of 48 hours or less, from sample collection to a result

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- Nicole Masters
- Emma Anastasi



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