



MLST FOR EPIDEMIOLOGICAL STUDY

Presented by Watsawan Prapasawat

Department of Clinic, Faculty of Veterinary Medicine

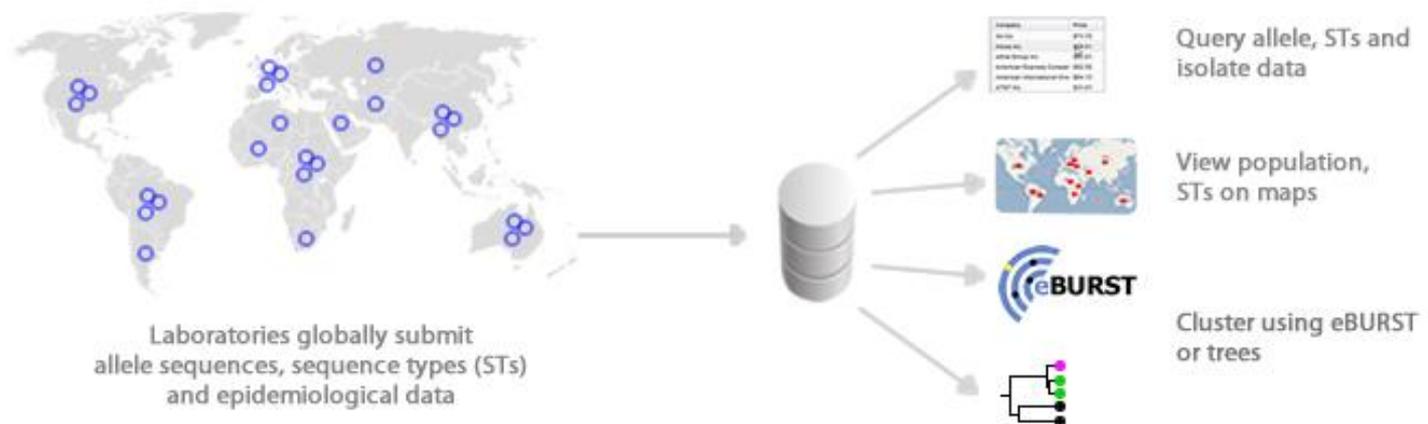
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Principle of MLST

- Multilocus sequence typing (MLST) is a procedure for **characterising isolates of bacterial species** using the **sequences of internal fragments of (usually) seven house-keeping genes**.
- MLST is based on PCR amplification and sequencing of internal fragments of a number (usually **6 or 7**) of essential or housekeeping genes spread around the bacterial chromosome.
- The **differences between the sequences** are used to define sequence types (**STs**).

The aim of MLST

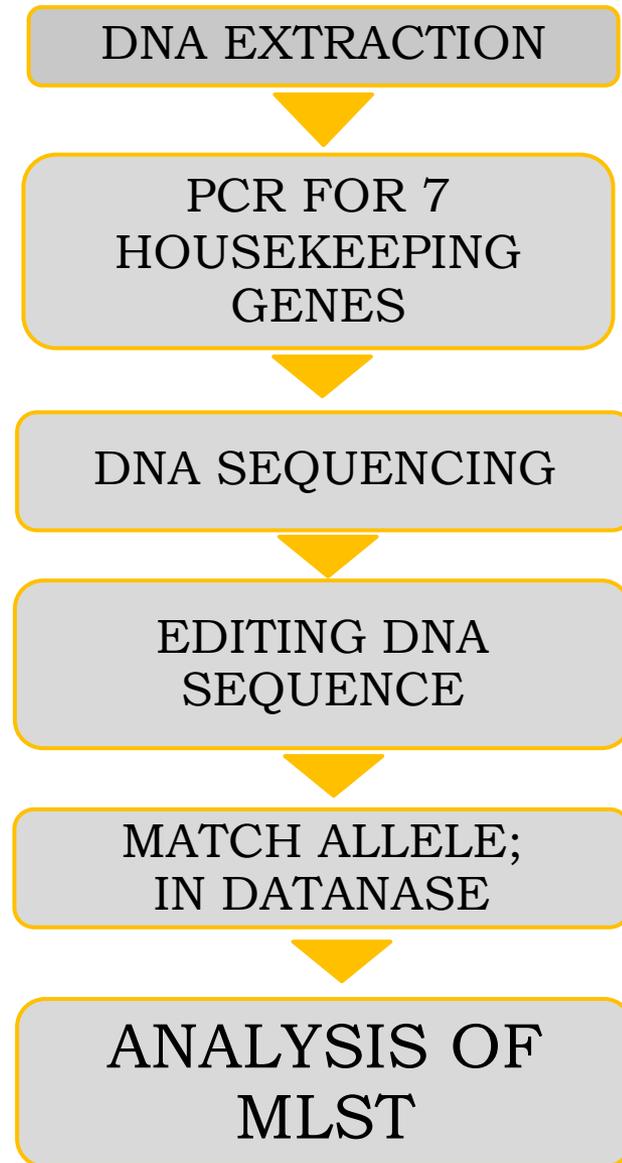
- It is provide a **portable, accurate, and highly discriminating typing** system that can be used for most bacteria and some other organisms.
- If the same genes were used in different studies, then the typing information of the isolates from these different studies may be compared and analyzed together.



Multilocus sequence typing (MLST)

- MLST schemes have been developed for the most important bacterial pathogens, including
 - ✓ *Streptococcus pyogenes*
 - ✓ *Haemophilus influenza*
 - ✓ *Staphylococcus aureus*
 - ✓ *Campylobacter jejuni*
 - ✓ *Enterococcus faecium*
 - ✓ *Escherichia coli*
 - ✓ *Salmonella* spp.

**Diagrammatic
representative
for procedure
used for MLST**



PCR primers of housekeeping genes of *E. coli* for MLST

Gene	Primer	Annealing (Temp)	Amplicon size (bp)
<i>adk</i>	ATTCTGCTTGGCGCTCCGGG (F) CCGTCAACTTTCGCGTATTT (R)	54	583
<i>fumC</i>	TCACAGGTCGCCAGCGCTTC (F) GTACGCAGCGAAAAAGATTC (R)	54	806
<i>gyrB</i>	TCGGCGACACGGATGACGGC (F) ATCAGGCCTTCACGCGCATC (R)	60	911
<i>icd</i>	ATGGAAAGTAAAGTAGTTGTTCCGGCACA (F) GGACGCAGCAGGATCTGTT (R)	54	878
<i>mdh</i>	ATGAAAGTCGCAGTCCTCGGCGCTGCTGGCGG(F) TTAACGAACTCCTGCCCCAGAGCGATATCTTTCTT (R)	60	932
<i>purA</i>	CGCGCTGATGAAAGAGATGA (F) CATACGGTAAGCCACGCAGA (R)	54	816
<i>recA</i>	CGCATTCGCTTTACCCTGACC (F) TCGTCGAAATCTACGGACCGGA (R)	58	780

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For citation please refer to: Wirth, T., Falush, D., Lan, R., Colles, F., Mensa, P., Wieler, L.H., Karch, H., Reeves, P. R., Maiden, M. C., Ochman, H., and Achtman M. 2006. [Sex and virulence in *Escherichia coli*: an evolutionary perspective.](#) *Mol. Microbiol.* **60**(5), 1136-1151.

Update information: ST complexes have been updated again on 17.05.2007. There are currently 600 STs and 54 ST complexes. The criteria have also been changed and are now groups of at least 3 STs sharing 6 alleles in pair-wise comparisons. The assignments of STs to some of the previous ST complexes have changed as a result, although we have tried to maintain consistency.

ST complexes have been updated again on 24.08.2005. Multiple new ST Complexes have been assigned and multiple STs have been assigned to known complexes. Due to the increased number of strains assigned to the ST29 Complex, it has become unclear whether these bacteria are closely related or only linked by one intermediate recombinant. Therefore, this has now been split into the ST23 and ST29 Complexes.

ST complexes have been updated on 23.11.2004. This includes the merging of ST21, 29 and 90 Complexes into ST29 Complex and ST3 and 17 Complexes into ST20 Complex. Multiple new ST complexes have been assigned. A number of STs have been merged with other STs due to curation of the database.

Protocols used for MLST of *Escherichia coli*

Genes

The *E. coli* MLST scheme uses internal fragments of the following seven house-keeping genes:

adk (adenylate kinase)
fumC (fumarate hydratase)
gyrB (DNA gyrase)
icd (isocitrate/isopropylmalate dehydrogenase)
mdh (malate dehydrogenase)
purA (adenylosuccinate dehydrogenase)
recA (ATP/GTP binding motif)

PCR Amplification

Please note: These include new primer sequences (added 26 July 2004; labelling corrected on 5 March, 2007) whose labels indicate the genomic direction rather than reading frame.

The primer pairs for the PCR amplification of internal fragments of these genes can be chosen from:

← → ↻ [mlst.warwick.ac.uk/mlst/dbs/Ecoli/documents/primersColi_html](#)

PCR Amplification

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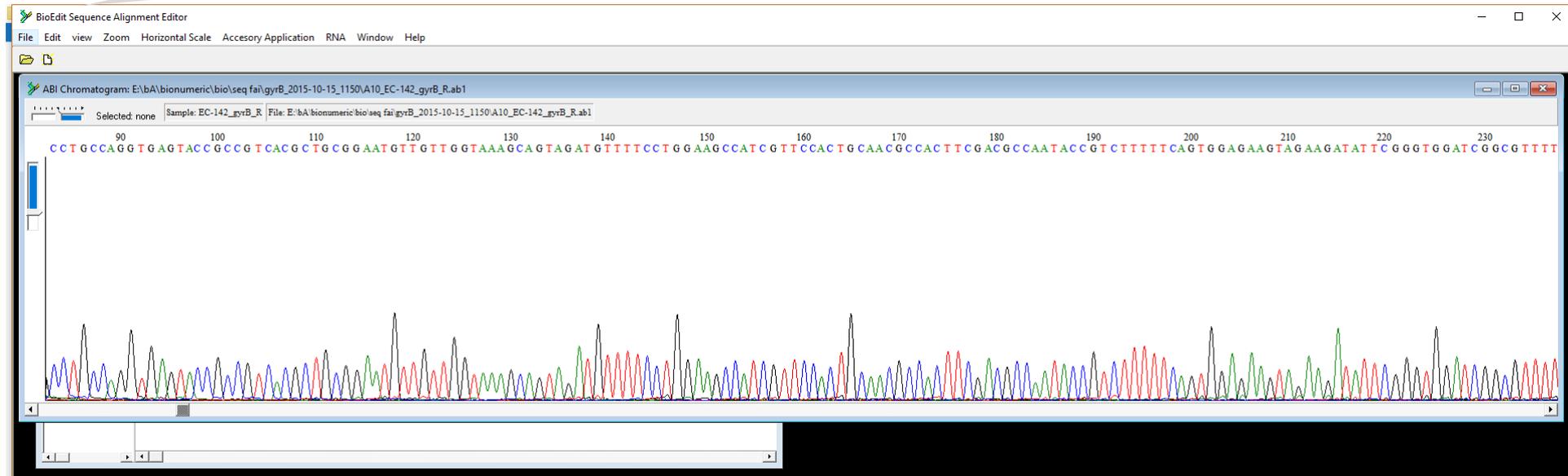
Gene:	Product length:	Annealing Temperature:
<i>adkF</i> 5'-ATTCTGCTTGCGCTCCGGG-3' <i>adkR</i> 5'-CCGTCAACTTTCGCGTATTT-3' <i>adkF1</i> 5'-TCATCATCTGCACTTTCCGC-3' <i>adkR1</i> 5'-CCAGATCAGCGCGAACTTCA-3'	583 bp	54° C
<i>fumCR1</i> 5'-TCCCGGCGAGATAAGCTGTGG-3' <i>fumCF</i> 5'-TCACAGGTCGCCAGCGCTTC-3' <i>fumCR</i> 5'-GTACGCAGCGAAAAAGATTC-3'	806 bp	54° C
<i>gyrBF</i> 5'-TCGGCGACACGGATGACGGC-3' <i>gyrBR1</i> 5'-GTCATGTAGCGTTCCAGGG-3' <i>gyrBR</i> 5'-ATCAGGCCTTCACGCGCATC-3'	911 bp	60° C
<i>icdF</i> 5'-ATGGAAGTAAAGTAGTTGTTCCGGCACA-3' <i>icdR</i> 5'-GGACGCAGCAGGATCTGTT-3'	878 bp	54° C
<i>mdhF</i> 5'-ATGAAAGTCGCAGTCCTCGGCGTGCTGGCGG-3' <i>mdhR</i> 5'-TTAACGAACCTCGCCAGAGCGATATCTTCTT-3' <i>mdhF1</i> 5'-AGCGCGTTCTGTTCAAATGC-3' <i>mdhR1</i> 5'-CAGGTTCAGAACTCTCTGT-3'	932 bp	60° C
<i>purAF1</i> 5'-TCGGTAACGGTGTGTGCTG-3' <i>purAF</i> 5'-CGCGCTGATGAAAGAGATGA-3' <i>purAR</i> 5'-CATACGGTAAGCCACGCAGA-3'	816 bp	54° C
<i>recAR1</i> 5'-AGCGTGAAGGTAACCTGTG-3' <i>recAF</i> 5'-CGCATTGCTTTACCCTGACC-3' <i>recAF1</i> 5'-ACCTTTGTAGCTGTACCAGC-3' <i>recAR</i> 5'-TCGTCGAAATCTACGGACCGGA-3'	780 bp	58° C

Conditions:

PCR: 2 min at 95°, 30 cycles of 1 min at 95°, 1 min at annealing temp, 2 min at 72° followed by 5 min at 72°. The PCR reaction contains 50 ng of chromosomal DNA, 20 pmol of each primer, 200 umol (10 ul of a 2 mM solution) of the dNTPs, 10 ul of 10x PCR buffer, 5 units of Taq polymerase and water to 100 ul.

Demonstrate for MLST

Prove sequence by Bioedit program



Allele template

Allelic profile of E. coli strain MG1655 (see Genebank)

adk (536 bp):
GGGAAAGGGAAGCTCAGGCTCAAGTTCAATCATGGGAAATATGGTATTCCGCAAACTCCACTGGCGATATGTCGGTG
CTGCGGGTCAAACTTGGCTCCGAGCTGGTAAACAGCAAAAGACATTATGGATGCTGGCAAACTGGTACCGGACGAA
CTGGTGATCGCGTGGTTAAAGAGCGCATTGCTCAGGAAGACTGCCGTAATGGTTTCTGTTGACGCGCTTCCGCGG
TACCATTCCGACAGGACAGCGGATGAAAGAGCGGGCATCAATGTTGATTACGTTTCCGAAATTCGACGATCCGCGAGC
AACTGATCGTTGACCGTATCGGTCGCGCGCTTCATGCCCGCTTGGTGGTATACACGTTAAATCAATCCG
CCGAAAGTAAAGGCAAAAGACGACGCTTACCGGTGAAGAACTGACTCCGTTAAAGATGATCAGGAAGAGACGCTGAC
TAAAGCTCTGGTTGAATACCATCAGATGACAGCAGCGCTGATCGCTACTACTCCAAAGAGCAGAAAGCGGGTA

fumC (469 bp):
CGAGCGCCATTCTGTCAGGCGCGGATGAAGTACTGCGACGACGACATGACGACGAATCCCGGCTGGTATCTGGCAG
ACCGGCTCCGGCACGCAAAAGTAAACATGAACATGAACGAAGTGC TGGCTAACCGGGCGAATGAACTCCGGCGGTG
CGCGGGATGGAACGAAAGTTACACCTAACGACGACGTGAACAAAAGCCAAAGTCCACAGATGCTTTCCGACGG
CGATGACAGTTGGCGGCTGCTGGCGCTGCGCAAGCAACTCATTCCAGCTTAAACCTGACACAGACAGCAATGAAT
GAGAAATCCCGTCTTTTCCGATATCGTCAAATTTGGTCTACTCATTCCAGGATGCCACGCCGTTAACCGCTGGG
GCAGGAGATTTCCGGCTGGGTAGCGATGCTCGAGCATAATCTCAACATATCGAATACAGCCTGCCCTACGTAAGCGG
AACTGCG

gyrB (460 bp):
GGTCTGCACGGCGTTGGTGTTCCTGGTAGTAAACGCCCTGTCCGCAAAAAGTGGAGCTGGTATCCAGCGCGAGGGTAA
AATTCACCTCGAGATCAGCAACGCGGTACCGAGCGCCCGCTGGCGTTACCGCGGAGACTGAAAAAAGCCGGCA
CCATGGTGGTTCCTGGCCAGCCTCGAAACCTTCAACATGACCGAGTTTCAATGAAATTCGGCGAAACGCT
CTGCGTAGGTTGCTTCCCTCACTCCGCGCTTCCATTCTGCTCGCGGACAAAGCCGACGGCGAAAGAACCACTT
CCACTATGAAGCGGGATCAAGGGCTTCTGTTGAATATCGAACAAAGCAAAAAGCCGATCCACCCGAATATCTTCT
ACTTCTCCACTGAAAAAGACGGTATTTGGCTCGAAGTGGCGTTGCGAGTGAACGATGCTTCCAGGAAAAACATCT

icd (518 bp):
CGACGCTCGAGTCGAAAGCCATAAAGGCGAGCGTAAATCTCCTGGATGAAATTTACCCGGTGAAAAATCCA
CACAGGTTATGGTCAGGACGCTTGGCTGCCGCTGAAACTTGTGATCTGTTGTAATCGGTTGCCATTAAA
GGTCCCGTGACCACTCCGGTTGGTGGCGTATTCGCTCTGAAAGTTCGCGTCCGCAAGAACTGGATCTACAT
CTGCGTCCGCTCCGGTACTTACTATCAGGCACTCCAAAGCCGGTAAACACCCCTGAATGACGATATGGTATTCT
TCCGTGAAACTCGGAAGACATTTATCGGGTATCGATGAAAGTCACTCTGCCGACGCCGAGAAAGTAAATA
TTCTCCGCTGAAGAGATGGCGTGAAGAAATTCGCTTCCCGGAACATTCGGGTATCGGTATTAAGCCGTTCTTGA
AGAAGGCCCAAACTGCTGGTTCGTGACGGATCGAATACGCAATTGCTAACGATC

mdh (452 bp):
GGCGTAGCGCGTAAACCGGGTATGGATGCTTCCGACCTGTTTAACTGAAACCGCGCATCGTAAAAACCTGGTACA
GCAAGTTGCGAAAACTGCCGAAAGCGGTGCATTGGTATTAACCTAACCCGCGTAAACACACAGTTGCAATTCGTC
CTGAAAGTCTGAAAAAGCCGGTGTATGACAAAACAACTGTTCCGGCTTACACGCTGGATCATCTGTTCC
AACACCTTTTGGGAAATGAAAGGCAAAACGACGCGAAGTGAAGTCCCGGTTATGGCGCTACTCTGGTGT
TACCATTCCGCGCTGCTGACAGGTTCTGGCGTATGTTTACGAGCAGGAAGTGGCTGATCTGACCAAAACGCA
TCCAGAACCGGGTACTGAAGTGGTTGAAGCGAAGCCGCTGGCGGGTCTGCAACCCTGCTATGGG

puA (478 bp):
ATAACGGCGGTGAGAAAGCGCGTGGCGGAAAGCGATCGGCACACCCGGTCTGGTATCGGGCTGCTTATGAAGAT
AAAGTAGCACGTCGCGGCTCGCTGTTGGCGACCTTTTCGACAAAGAAACCTTCGCTGAAAACTGAAAGAAAGTGA
GGAATATCACAACCTCCAGTTGGTTAACTACAAAGCTGAGAGCGGTTGATTACAGAAAGTTCTGGATGATACGA
TGGCTGTTGGCGACATCCGACTTATGTTGGTTGACGTTTGTGACCTCTCGACAGCGCCGCTCAGCGTGGCGAT
TTGCTCATGTTTGAAGGTGCGCAGGGTACGCTGCTGGATTCGACACGCTACTTATCCGTAACCTTCTCCAA
CACCACTGCTGGTGGCGTGGCGACCGGTTCCGGCTGGGCGCGGTTATGTTGATACGTTCTGGGTATCTCAAAG
CTTACTCCACTCGTG

recA (510 bp):
CGCACGTAACCTGGGCGTGCATATCGACAACCTGCTGTGCTCCAGCGGACACCGGCGAGCAGGCACTGAAATCT
GTGACGCCCTGGCGCGTTCGGCCAGTAGACGTTATCGTGTGACTCCGTTGGCGGCACTGACGCCGAAAGCGGAA
ATCGAAGCGGAAATCGCGACTCTACATGGGCTTGGCGCACATGATGAGCGAGCGATCGTAAGCTGGCGGG
TAACTGAAACAGTCCAAACGCTGCTGATCTCATAAACAGATCCGATGAAATTTGGTGGTGGTGGTAAAC
CGGAACCACTACCGTGGTAAACGCGTAAATCTACGCTCTGTTCTCGACATCCGCTGATCGGCGGGT
AAAAGGGCGAAAACTGGTGGTAGCGAAACCGGCTGAAAGTGGTGAAGAACTGCTGCGCCGTTAAAAA
GGCTGAATCCAGATCCTTACGGCAAGGTATCAACTTCTACGGCGA

BioEdit Sequence Alignment Editor - [CAP contig assembly program Output]
File Edit Sequence Alignment View Accessory Application RNA World Wide Web Options Window Help
Courier New 11 B 2 total sequences
Mode: Select / Slide Selection: null Position: 1: cap_adk1 536 b 26 Sequence Mask: None Numbering Mask: None Start ruler at: 1
cap adk1 T T C A T C A T G G A G A A A T A T G G T A T T C C G C A A A T C T C C A C T G G C G A T A T G T G C G T G C G T G C G G T C A A A T C T G G C T C C G A G C T G G G T A A A C A A G C A A A A G C
cap_Contig-0 T T C A T C A T G G A G A A A T A T G G T A T T C C G C A A A T C T C C A C T G G C G A T A T G T G C G T G C G T G C G G T C A A A T C T G G C T C C G A G C T G G G T A A A C A A G C A A A A G C

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Escherichia coli MLST Database.

adk	fumC	gyrB	icd	mdh	purA	recA
<input type="text" value="5"/>	<input type="text"/>					

Sequence:

```
GGGGAAAGGGACTCAAGCTCAGTTCATCATGSAAGAAATG6GTATTCCGCAAACTCCACTGSCSA  
TATGCTGCCTGCTGCGGTCAAATCTG9GCTCC8AGCTGGGTAAACAA8CAAAAAGACATTAT9GAT9C  
TGGCAAACTGGTCACCGACGAACGTGGTATC8CGCTGGTAAAGAGCGCATTGCTCAG9AAAGACTG  
CCGTAAATGGTTTCTGTGGACGGCTTCCCGCGTACATTCCGCA8GGCAGACGCGATGAAAAGAAC  
GGGCATCAATGTTGATTACGTTCTG9AATTG8ACGTACCGACGAACTGATTGTTGATCGTATCGT  
A8GCCCGCCGCTCATGCGCCGCTG9TGTGTTTATCACGTAAATCAATCGCCGAAAAGTAGA  
A8GCAAAAAGACGAGGTTACCGGTGAAGAACTGACTACCGTAAAGACGATCAG9AAAGAAACCGT9CG  
TAAAAGCTCTGGTGAATACCATCAGATGACTGCACCGCTGATCGGCTACTACTCCAAAAGAACG9SA  
AGCGGGTA
```

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Gene Fragment

adk ▼

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Allele number for *adk* gene

Escherichia coli MLST Database.

<i>adk</i>	<i>fumC</i>	<i>gyrB</i>	<i>icd</i>	<i>mdh</i>	<i>purA</i>	<i>recA</i>
6						

Sequence:

```
GGGGAAAGGGACTCAGGCTCAGTTCATCATGGAGAAATATGGTATTCC
GCAATCTCCACTGGCGATATGCTGCGTGCTGCGGTCAAATCTGGCTC
CGAGCTGGGTAAACAAGCAAAGACATTATGGATGCTGGCAAATGGT
CACCGACGAACTGGTGATCGCGCTGGTTAAAGAGCGCATTGCTCAGGA
AGACTGCCGTAATGGTTTCCTGTTGGACGGCTCCCGCGTACCATTCC
GCAGGCAGACGCGATGAAAGAAGCGGGCATCAATGTTGATTACGTTCT
GGAATTCGACGTACCGGACGAACTGATTGTTGATCGTATCGTAGGCCG
CCGCGTTCATGCGCCGCTCTGGTCGTGTTTATCAGTTAAATTCATCC
GCCGAAAGTAGAAGGCAAAGACGACGTTACCGGTGAAGAACTGACTAC
CCGTAAGACGATCAGGAAGAAACCGTGCCTAACGCTCTGGTTGAATA
```

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Gene Fragment:

adk

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Submit Forward and

Reward sequence for generating new allele

type

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Escherichia coli MLST Database.

adk	fumC	gyrB	icd	mdh	purA	recA
<input type="text"/>						

Sequence:

```

CGAGCGCCAT TCGTCAAGCG GCGGATGAAG TACTGSCAGG ACAGCATGAC
GACGAATTCC CGCTGGCTAT CTGSCAGACC GGCTCCGSCA CGCAAAGTAA
CATGAACATG AACGAAGTGC TGGCTAACCG GGCCAGTSA TACTCGCGG
GTGTGCGCGG GATGGAACGT AAAGTTCACC CTAACGACGA CGTGAACAAA
AGCCAAAAGT CCAACGATGT CTTTCGACG GCGATGACGA TTGCGGCGCT
GCTGGCGCTG CGCAAGCAAC TCATTCTCA GCTTAAAAAC CTGAGACAGA
CACTGAATGA GAAATCCCGT GCTTTTGCCG ATATCGTCAA AATTGGTCGT
ACTCACTTGC AGSATGCCAC GCCGTTAACG CTGGGCGAGG AGATTTCCGG
CTGGGTAGCG ATGCTCGAGC ATAATCTCAA ACATATCGAA TACAGCTGC
CTCACGTAGC GGAACTGGC
  
```

Your sequence with length: 469 most resembles FUNC11 starting at 1 and with 468/469 matches

```

CGAGCGCCATTGTCAGGCGCGGATGAAGTACTGGCAGSACAGCATGACGACGAATTCC
|||||
CGAGCGCCATTGTCAGGCGCGGATGAAGTACTGGCAGSACAGCATGACGACGAATTCC
|||||
CGCTGGCTATCTGSCAGACC GGCTCCGSCACGCAAAGTAAACATGAACATGAACGAAAGTGC
|||||
CGCTGGCTATCTGSCAGACC GGCTCCGSCACGCAAAGTAAACATGAACATGAACGAAAGTGC
|||||
TGGCTAACCGGSCCAGTGAATTACTCGGCGGTGTGCGCGGAGTGAACGTAAGTTCACC
|||||
TGGCTAACCGGSCCAGTGAATTACTCGGCGGTGTGCGCGGAGTGAACGTAAGTTCACC
|||||
CTAACGACGACGTGAACAAAAGCCAAAAGTCCAACGATGTCTTCCGACGGCGATGACAG
  
```

Get Info

Gene Fragment

fumC

Submit new Sequence

Please upload at least 2 supporting .ABI, .SCF or .ESD files for this sequence!

<input type="button" value="Choose File"/> No file chosen	<input type="button" value="Choose File"/> No file chosen	<input type="button" value="Choose File"/> No file chosen
<input type="button" value="Choose File"/> No file chosen	<input type="button" value="Choose File"/> No file chosen	

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- Mark Achtman
mksmaj@live.warwi

Options

With attachments

Curation of your submitted sequence(s) for Ecoli MLST

m.achtman@warwick.ac.uk
Mon 9/7/2015, 3:51 PM
You; m.achtman@warwick.ac.uk

Policy on MLST alleles a...
32 KB

Download Save to OneDrive - Personal

Dear watsawan ,

You submitted traces and sequence for **mdh411** (07 September, 2015).
After curation, we are pleased to acknowledge that the traces support the
uploaded sequence and this (these) allele has now been permanently accepted in the database.

We append a document with details on privacy and our criteria for accepting
novel alleles and MLST patterns for your information.

Thank you very much for your contribution. Please feel free to contact me
if there are any questions.

Mark Achtman
Warwick Medical School, University of Warwick
m.achtman@warwick.ac.uk

Escherichia coli MLST Database.

ST	adk	fumC	gyrB	icd	mdh	purA	recA	ST Complex
ST48	<input type="text" value="6"/>	<input type="text" value="11"/>	<input type="text" value="4"/>	<input type="text" value="8"/>	<input type="text" value="8"/>	<input type="text" value="8"/>	<input type="text" value="2"/>	ST10 Cplx
ST48	6	11	4	8	8	8	2	ST10 Cplx
ST880	6	11	4	8	11	8	2	None
ST832	43	11	4	8	8	8	2	None
ST772	6	11	4	149	8	8	2	None
ST735	92	11	4	8	8	8	2	None

Number of:

related STs

Find ST

identical alleles

Submit new Strain

Sequence:

[Empty text area for sequence input]

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Gene Fragment:

adk ▼

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unknown ST

No ST Complex

adk	fumC	gyrB	icd	mdh	purA	recA
6	19	3	16	9	8	341

*STRAIN :	<input type="text" value="EC-R1"/>
*HOST_TYPE :	<input type="text" value="Animal"/>
*HOST_SPECIES :	<input type="text" value="Swine"/>
*PATHORNONPATH :	<input type="text" value="Pathogenic"/>
YEAR :	<input type="text" value="2014"/>
CITY :	<input type="text"/>
*COUNTRY :	<input type="text" value="Thailand"/>
*CONTINENT :	<input type="text" value="Asia"/>
*SOURCE_LAB :	<input type="text" value="Mahidol University"/>
PATHOGEN_TYPE :	<input type="text" value="Escherichia coli"/>
SEROTYPE :	<input type="text"/>
DISEASE :	<input type="text" value="Diarrhoea"/>
SIMPLE_DISEASE :	<input type="text" value="Diarrhoea"/>
SIMPLE_PATHOGENICITY :	<input type="text" value="ETEC"/>
MLEE_ECOR_CLUSTER :	<input type="text"/>
SEROLOGICAL_GROUP :	<input type="text"/>
OMP_PATTERN :	<input type="text"/>

Escherichia coli is already present in the database and cannot be reentered
Please fill in information STRAIN

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Show column selector

Showing records 7739 to 7758 of 7758 go to 7739 Refresh

Strain	adk	fumC	gyrB	icd	mdh	purA	recA	ST	Host Type	Host Species	Pathornonpath	Country	Continent	Source Lab
J4747747	6	4	12	1	63	18	7	5206	Companion animal	Dog	Pathogenic	U.S.A.	North America	Veterinary medicine pharmacology laboratory
729-121114-1-16 salp	6	295	12	18	11	8	7	5207	Poultry	Chicken	Pathogenic	Denmark	Europe	Veterinary Disease Biology, KU
723-081214-16n	10	7	4	8	12	8	194	5208	Poultry	Chicken	nonpathogen	Denmark	Europe	Veterinary Disease Biology, KU
730-110714-11n	6	95	4	88	7	32	7	5209	Poultry	Chicken	Non pathogen	Denmark	Europe	Veterinary Disease Biology, KU
723-021014-16s	6	11	4	8	8	32	2	5210	Poultry	Chicken	Non pathogen	Denmark	Europe	Veterinary Disease Biology, KU
723-081214-28s	6	7	4	1	31	28	2	5211	Poultry	Chicken	Non pathogen	Denmark	Europe	Veterinary Disease Biology, KU
2013BD34	14	14	10	499	17	7	10	5212	Mammal	Homo sapiens	Pathogen	Korea	Asia	Kyung Hee University Hospital
2014BD45	53	637	47	13	36	28	29	5213	Human	Human	Pathogen	Korea	Asia	Kyung Hee University Hospital
WCHECY0402	35	183	433	25	4	5	73	5214	Human	Human	Pathogen	China	Asia	ZONG ZHIYONG, WEST CHINA HOSPITAL, SICHUAN UNIVERSITY
EC02	382	11	4	8	8	8	2	5215	Human	Human	unknown	China	East Asia	the second hospital of anhui medical UNIVERSITAS
EC05	10	4	12	8	12	18	7	5216	Human	Human	pathogen	China	East Asia	the second hospital of anhui medical UNIVERSITAS
723-081214-35s	6	638	12	1	20	12	7	5217	Poultry	Chicken	Non pathogen	Denmark	Europe	Veterinary Disease Biology, KU
L2017061	88	103	19	73	23	44	26	5219	Companion animal	Dog	Pathogenic	U.S.A.	North America	Veterinary medicine pharmacology laboratory
L4898741	10	11	4	8	41	8	7	5220	Companion animal	Dog	Pathogenic	U.S.A.	North America	Veterinary medicine pharmacology laboratory
Escherichia coil	6	4	4	18	11	8	7	5221	Human	Human	unknown	China	Asia	Veterinary Pharmacology Laboratory of South China Agricultural University
EF201410-17	64	11	1	8	25	18	343	5222	Faeces	Swine	Unknown	China	Asia	Shanghai Municipal Center for Disease Control & Prevention
EC14040	83	172	142	158	399	2	342	5223	Faeces	Human	Unknown	China	Asia	Shanghai Municipal Center for Disease Control & Prevention
EC14072	6	11	4	8	7	8	2	5224	Faeces	Human	Unknown	China	Asia	Shanghai Municipal Center for Disease Control & Prevention
EC14039	428	639	434	517	400	373	344	5225	Faeces	Human	Unknown	China	Asia	Shanghai Municipal Center for Disease Control & Prevention
EC-R1	6	1	3	16	9	8	341	5226	Animal	Swine	pathogen	Thailand	Asia	Mahidol University

Showing records 7739 to 7758 of 7758 go to 7739 Refresh

The whole table of selected columns can be downloaded as CSV file by right-clicking this link: [Strain Ecoli.csv](#)

Note: some columns like STRAIN, are likely to be interpreted as date values by Excel. To prevent this, mark the column and change the format to text. (mem format cells)

Logged in as: watsawan

Website and databases managed by Mark Achtman.
Modified by Umakumar Veludhathan and Zhenzhou Zhou

This server is hosted at the University of Warwick.
Development funded by the BBSRC.

Analysis of MLST

- Allele-based method
- Nucleotide-based method

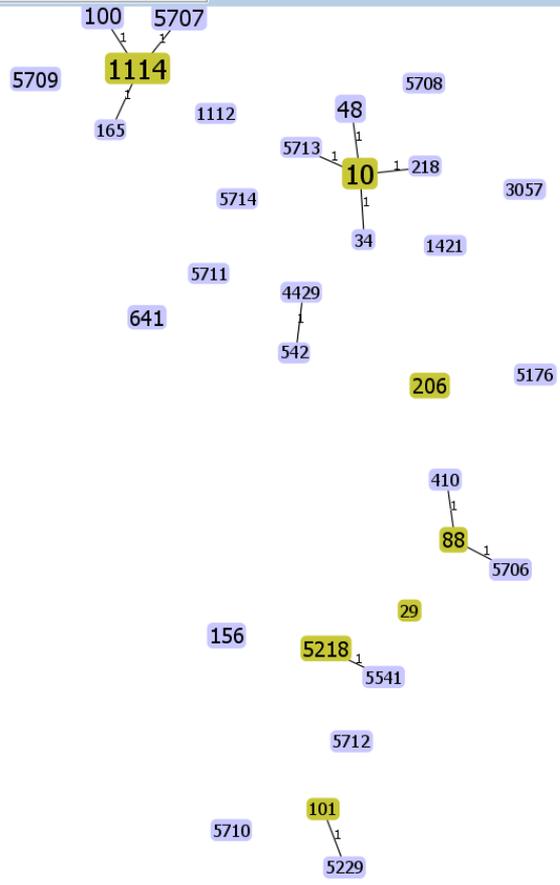
- m1st
 - Isolate Data
 - Multi-Locus Sequence Typing (MLST)
 - goeBURST Full MST (goeBURST distance)

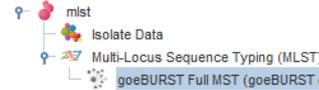
Groups

- 1

Level: |
Get Groups
Save Groups

Options [camera icon] [play icon] animation speed >>> 1





Groups

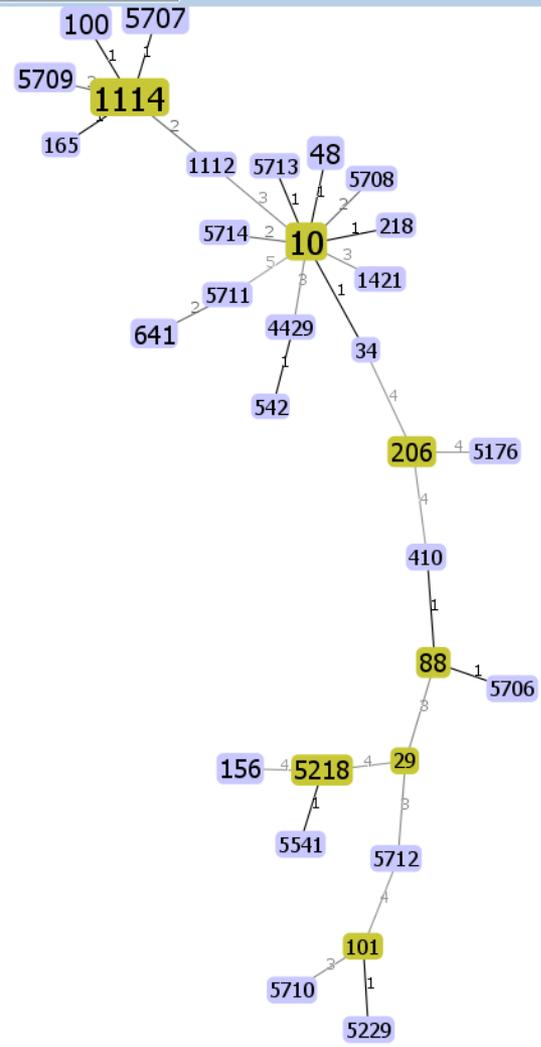
1

Level: 1

Get Groups

Save Groups

Options animation speed >> 1



Nucleotide-based method

Algorithm	Functionality	Link
MAFFT	Sequence alignment	http://mafft.cbrc.jp/alignment/software/
MAUVE	Sequence alignment	http://asap.ahabs.wisc.edu/mauve/index.php
JModeltest2	Selection of models of nucleotide substitution	https://code.google.com/p/jmodeltest2/
RAxML	ML inference of evolutionary relationships	http://www.exelixis-lab.org/
GARLI	ML inference of evolutionary relationships	http://code.google.com/p/garli/
PHYML	ML inference of evolutionary relationships	http://code.google.com/p/phyml/
MrBayes	Bayesian inference of evolutionary relationships	http://mrbayes.sourceforge.net/
BEAST	Bayesian inference of evolutionary relationships	http://beast.bio.ed.ac.uk/Main_Page
ClonalFrame	Bayesian inference of clonal relationships considering recombination	http://www.xavierdidelot.xtreemhost.host/clonalframe.htm

Mega5

<http://www.megasoftware.net/mega5/>

Nucleotide-based method

MEGA 7.0.20(7160929-x86_64)

File Analysis Help

Align Data Models Distance Diversity Phylogeny User Tree Ancestors Selection Rates Clocks Diagnose



M7: Analysis Preferences

Options Summary

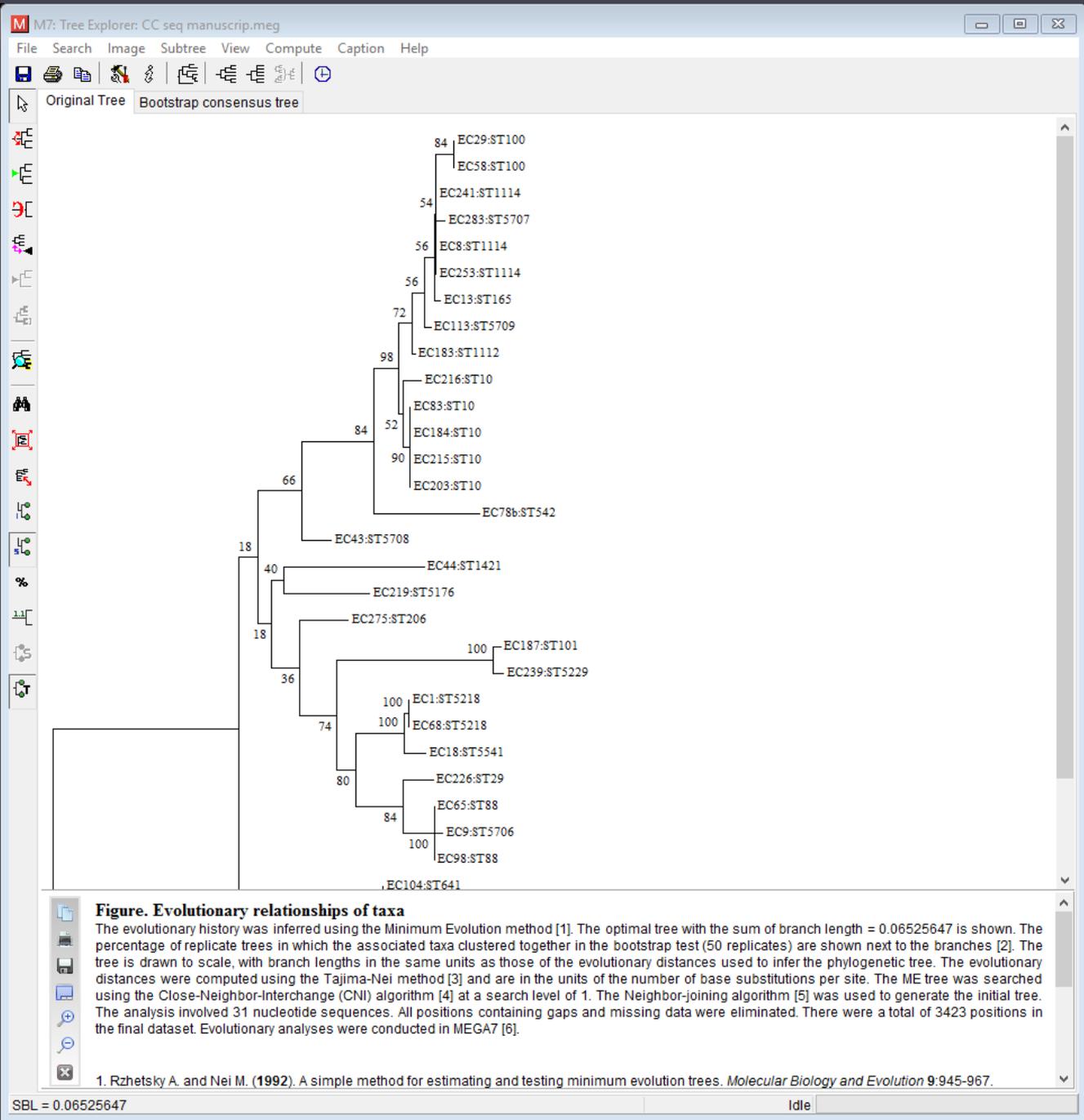
Option	Selection
Analysis	Phylogeny Reconstruction
Statistical Method	Maximum Likelihood
Phylogeny Test	
Test of Phylogeny	Bootstrap method
<i>No. of Bootstrap Replications</i>	1000
Substitution Model	
Substitutions Type	Nucleotide
Model/Method	Tamura-Nei model
Rates and Patterns	
Rates among Sites	Uniform rates
<i>No. of Discrete Gamma Categories</i>	Not Applicable
Data Subset to Use	
Gaps/Missing Data Treatment	Complete deletion
<i>Site Coverage Cutoff (%)</i>	Not Applicable
Tree Inference Options	
ML Heuristic Method	Nearest-Neighbor-Interchange (NNI)
Initial Tree for ML	Make initial tree automatically (Default - NJ/BioNJ)
<i>Initial Tree File</i>	Not Applicable
Branch Swap Filter	None
System Resource Usage	
Number of Threads	1

? Help Compute Cancel

Novel ST	Closest match ST	Variant locus	Fragment size (bp)	Aligned position	Nucleotide	Genetic event
5713	10	<i>icd8 – icd426</i>	518	206	C → T	Single mutation ¹
5706	88	<i>mdh20 - mdh411</i>	452	334	G → C	Single mutation
5707	1114	<i>fumC27 - fumC736</i>	496	232	G → T	Single mutation

¹Mutation: The variant locus differing by a small number of nucleotides and were not observed elsewhere in the database as part of another ST

S110:icd8 S15713:icd426	CGACGCTGCAGTCGAGAAAAGCCTATAAASGGGAGCGTAAAAATCTCCT	S188:mdh20 S15706:mdh411	GGTGTAGCGCGTAAACCGGGTATGGATCGTTCGGACCTGTTTAAACGTTAA	S1114:lumC27 S15707:lumC736	CGAGCGCCATTGCTCAGGCGGGGATGAAAGTACTGGCAGGACAGCATGAC
S110:icd8 S15713:icd426	TGGAAATTTACACCGGTGAAAAATCCACACAGGTTTATGGTCAGGAC	S188:mdh20 S15706:mdh411	AAGCGTGCATTGGTATTATCACTAACCCGGTTAACACCACAGTTGCGATT	S1114:lumC27 S15707:lumC736	GACGAATCCCAGCTGGCTATCTGGCAGACCGGCTCCGGCACGCCAAAGTAA
S110:icd8 S15713:icd426	TGGCCTGCCTGCTGAAACTCTTGATCTGATTCGTGAATATCGCGTTGC	S188:mdh20 S15706:mdh411	CGCGGTCACACGCTGGATATCATTGTTCCAAACACTTTT	S1114:lumC27 S15707:lumC736	CATGAACATGAACGAAGTCTGGCTAACCGGGCCAGTGAATTACTCGGGC
S110:icd8 S15713:icd426	TAAAGTCCGCTGACCACTCCGGTTGGTGGCGGATTCGCTCTCTGA	S188:mdh20 S15706:mdh411	TGAAAGCAACAGCCAGGGGAAGTTGAAGTCCGGTTAT	S1114:lumC27 S15707:lumC736	GTGTGGCGGGATGGAACGTAAAGTTCAACCCTAACGACGACGTGAACAAA
S110:icd8 S15713:icd426	TTGCCCTGCGCCAGGAAGTGGATCTCTACATCTGCCTGCCTCCGGTA	S188:mdh20 S15706:mdh411	TCTGGTGTACCAATCTGCCGCTGCTGTCACAGGTTTCCTG	S1114:lumC27 S15707:lumC736	AGCCAAAGTTCCAACGATGCTTTCCGACGGGATGACAGGTTGGGGGCT
S110:icd8 S15713:icd426	TACTATCAGGGCACTCCAAGCCCGGTTAAACACCCTGAACTGACCGA	S188:mdh20 S15706:mdh411	TACCGAGCAGGAAGTGGTGTCTGACCAAAGGATCCAG	S1114:lumC27 S15707:lumC736	GCTGGCGCTGGCCAAAGCAACTATTGCTCAGGTTAAACCCCTGCACACAGA
S110:icd8 S15713:icd426	GGTTATCTTCCGTGAAAAGTCCGGAAGACATTTATCGGGATCGAAT	S188:mdh20 S15706:mdh411	CTGAGGTGGTTGAAAGCAAGGCCGGTGGCGGGTCTGCAAC	S1114:lumC27 S15707:lumC736	CACTGAATGAGAAATCCCGTGCTTTTGCCGATATCGTCAAAATTTGGTCGT
S110:icd8 S15713:icd426	AAGCAGACTCTGCCGACCGGAGAAAAGTSAATTAATTCCTCGGTGAA	S188:mdh20 S15706:mdh411	GG	S1114:lumC27 S15707:lumC736	ACTCACTTGCAAGGATGCCACGCCGTTAACGCTGGGGCAGGAGATTTCCGG
S110:icd8 S15713:icd426	ATGGGGGTGAAGAAAATTCGCTTCCCGGAACATTGTGGTATCGGAT			S1114:lumC27 S15707:lumC736	CTGGATAGCGATGCTCGAGCATAATCTCAAACATATCGAATACAGCCTGC
S110:icd8 S15713:icd426	GCCGTGTTCCGGAAGAAAGCCACCAACGCTCGGTTCTGCGCAGCGATCGAAT			S1114:lumC27 S15707:lumC736	CTCACGTAAGCGAACTGGC
S110:icd8 S15713:icd426	ACGCAATTGCTAACGATC				



Application of MLST

- Molecular epidemiology and public health
- Population structure and dynamics
- Other applications

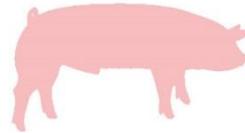
Virulence gene profiles, antimicrobial resistance patterns and phylogenetic relationship of *Escherichia coli* isolated from diarrheal and healthy weaning piglets and their environments in swine farm



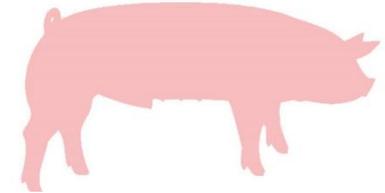
Introduction: Main causes according to pig's age



AT BIRTH-SUCKLING
1-4 WEEKS



WEANING
5-9 WEEKS



GROWING PIG - SLAUGHTER
10 WEEKS - 6 MONTHS

DISEASES: Iron deficiency, Arthritis, Atrophic Rhinitis, Aujeszky disease, Clostridia, Coccidiosis, **Post weaning**
Aujeszky disease, Clostridia, **diarrheal, Colibacillosis**, Epidemic diarrhea, PRRS, FMD, Swine dysentery, TGE,
Coccidiosis, **Diarrhe** virus, Classical swine fever etc.
Colibacillosis, Epidemic
POST WEANING DIARRHEA
PRRS, Salmonellosis,
Swine dysentery, TGE

CAUSE: Trauma, Vitamin deficiency Trauma, Vitamin deficiency, Viral infection, Bacterial infection
Viral infection,
Bacterial infection

EFFECTS: Morbidity, Mortality, Stunt grc Morbidity, Mortality, Stunted and grow slowly

ECO- LOSS Depend on the diseases Depend on the diseases

General Objective

- To analyze the phenotype and genotype of diarrheagenic *E. coli* isolated from weaning piglets with and without diarrhea and their environments in a swine farm

Schematic process

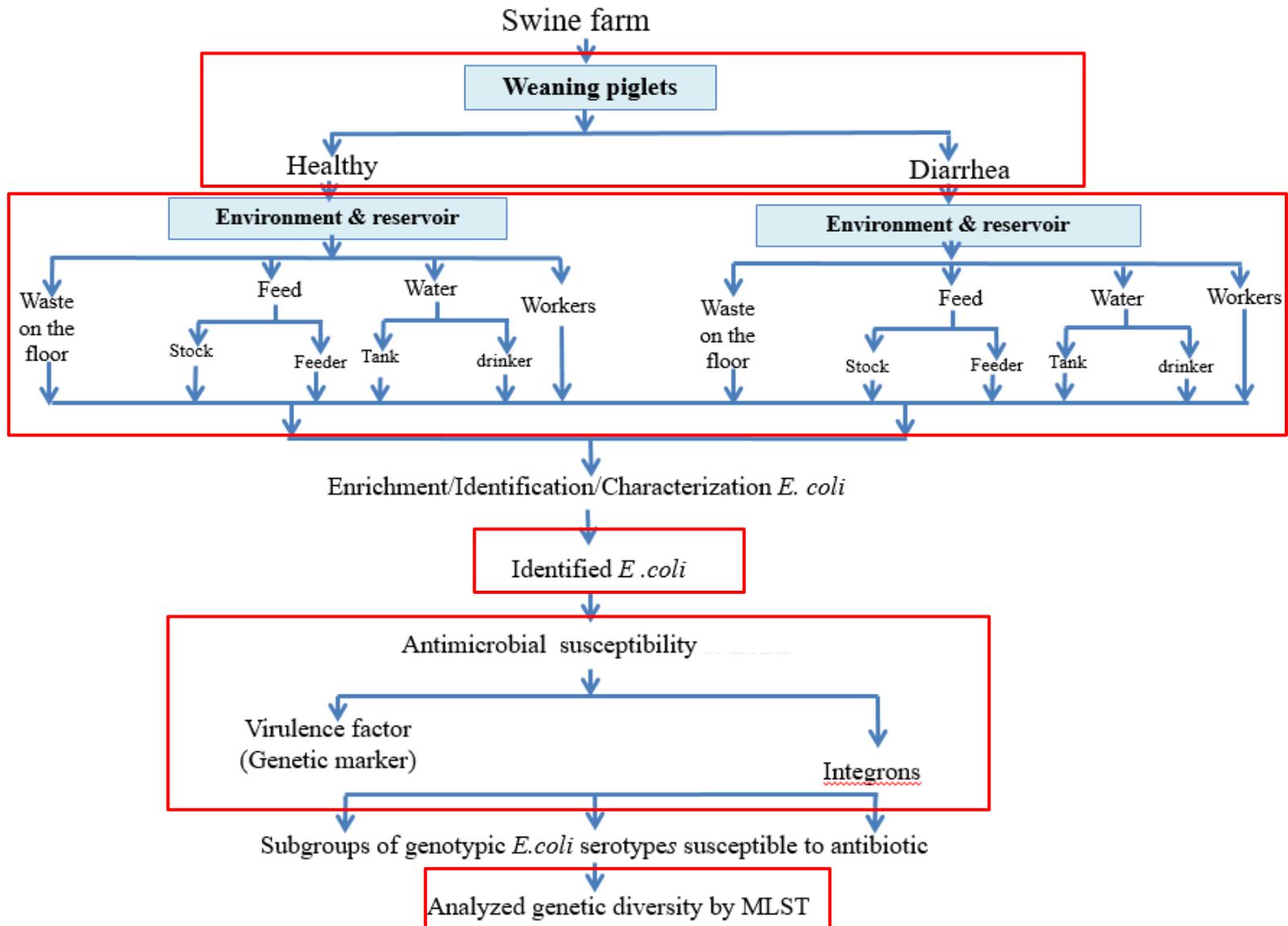


Table 5.3 Detection rate of *Escherichia coli* positive for virulence genes detected in diarrheal and healthy weaning piglets and environmental samples in swine farm at Central region, Thailand

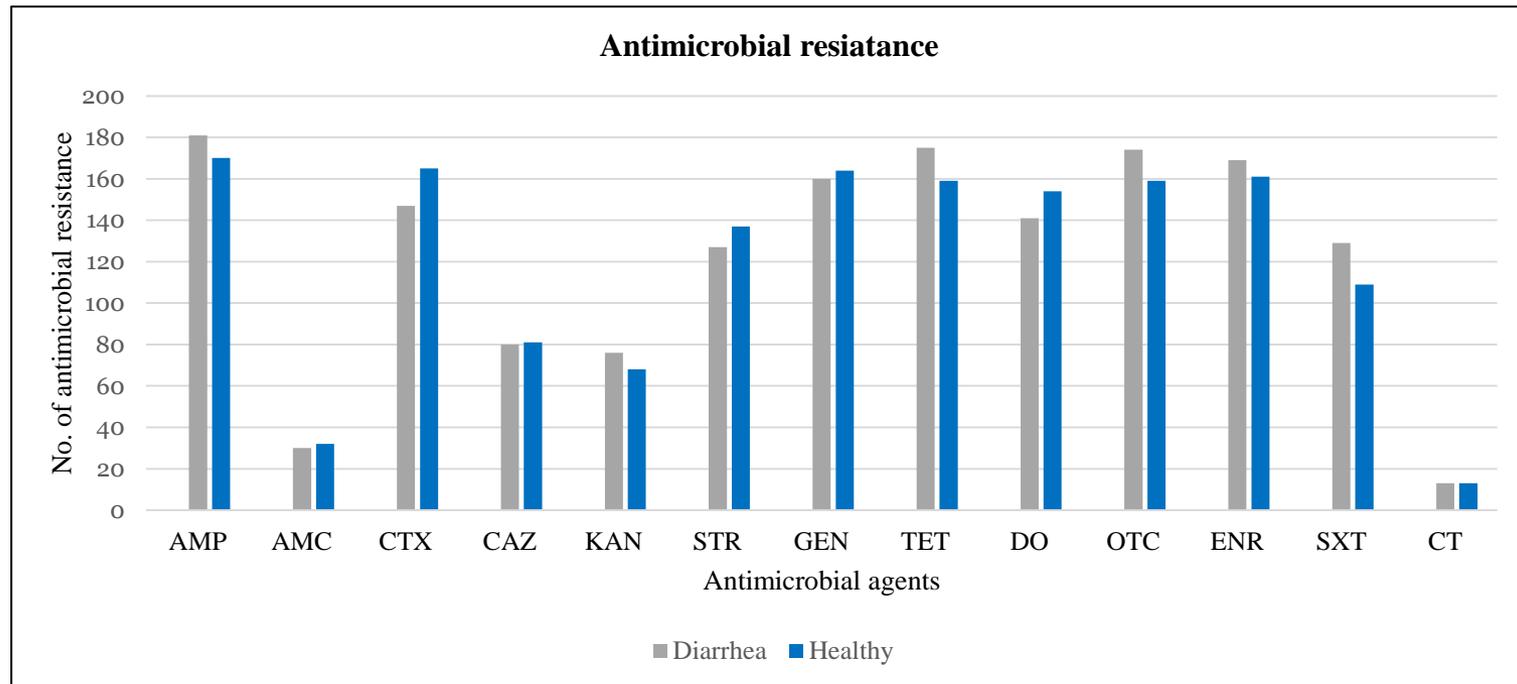
Sampling source	Total No. of positive <i>E. coli</i>	No. (%)	
		Pathogenic <i>E. coli</i>	Non pathogenic <i>E. coli</i>
Diarrheal piglet pens	182	68 (37.4)	114 (62.6)
Rectal swab	130	61 (46.9)	69 (53.1)
Feed from feeder	20	3 (15.0)	17 (85.0)
Water from nipple	12	0	12 (100.0)
Waste swab	16	2 (12.5)	14 (87.5)
Worker's hand	4	2 (50.0)	2 (50.0)
Healthy piglet pens	170	47 (27.6)	123 (72.4)
Rectal swab	116	35 (30.2)	81 (69.8)
Feed from feeder	20	4 (20.0)	16 (80.0)
Water from nipple	9	1 (11.1)	8 (88.9)
Waste swab	21	7 (33.3)	14 (66.7)
Worker's hand	4	0	4 (100.0)
Total	352	115 (32.7)	237 (67.3)

Table 5.6 Detection rate of antimicrobial resistance from diarrheal and healthy weaning piglets and environmental samples in swine farm at Central region, Thailand

Sampling source	Total no.	No. (%) of antimicrobial resistance of <i>E. coli</i> isolates												
		AMP	AMC	CTX	CAZ	KAN	STR	GEN	TET	DO	OTC	ENR	SXT	CT
Diarrheal pens	182	181 (99.5)	30 (16.5)	147 (80.8)	80 (44.0)	76 (41.8)	127 (69.8)	160 (87.9)	175 (96.2)	141 (77.5)	174 (95.6)	169 (92.9)	129 (70.9)	13 (7.1)
Rectal swab	130	130 (100.0)	19 (14.6)	104 (80.0)	57 (43.8)	53 (40.8)	88 (67.7)	113 (86.9)	128 (98.5)	95 (73.1)	127 (97.7)	119 (91.5)	98 (75.4)	6 (4.6)
Feed from feeder	20	20 (100.0)	1 (5.0)	15 (75.0)	7 (35.0)	10 (50.0)	17 (85.0)	17 (85.0)	10 (50.0)	10 (50.0)	10 (50.0)	10 (50.0)	10 (50.0)	0
Water from nipple	12	12 (100.0)	1 (8.3)	12 (100.0)	1 (8.3)	12 (100.0)	12 (100.0)	12 (100.0)	12 (100.0)	12 (100.0)	12 (100.0)	12 (100.0)	12 (100.0)	1 (8.3)
Waste swab	16	16 (100.0)	1 (6.2)	16 (100.0)	1 (6.2)	16 (100.0)	16 (100.0)	16 (100.0)	16 (100.0)	16 (100.0)	16 (100.0)	16 (100.0)	16 (100.0)	5 (31.3)
Worker's hand	4	4 (100.0)	1 (25.0)	4 (100.0)	1 (25.0)	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	4 (100.0)	1 (25.0)
Healthy pens	170	131 (77.1)	62 (36.5)	117 (68.8)	61 (35.9)	54 (31.8)	144 (84.7)	144 (84.7)	144 (84.7)	144 (84.7)	144 (84.7)	144 (84.7)	138 (81.2)	13 (7.6)
Rectal swab	116	116 (100.0)	18 (15.5)	116 (100.0)	18 (15.5)	116 (100.0)	116 (100.0)	116 (100.0)	116 (100.0)	116 (100.0)	116 (100.0)	116 (100.0)	116 (100.0)	8 (6.9)
Feed from feeder	20	20 (100.0)	2 (10.0)	20 (100.0)	2 (10.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	20 (100.0)	1 (5.0)
Water from nipple	9	9 (100.0)	5 (55.6)	9 (100.0)	8 (88.9)	7 (77.8)	9 (100.0)	9 (100.0)	8 (88.9)	8 (88.9)	8 (88.9)	9 (100.0)	9 (100.0)	2 (22.2)
Waste swab	21	21 (100.0)	7 (33.3)	19 (90.5)	11 (52.4)	9 (42.9)	18 (85.7)	20 (95.2)	20 (95.2)	20 (95.3)	21 (100.0)	21 (100.0)	16 (76.2)	2 (9.5)
Worker's hand	4	4 (100.0)	2 (50.0)	4 (100.0)	2 (50.0)	3 (75.0)	4 (100.0)	4 (100.0)	4 (100.0)	2 (50.0)	4 (100.0)	2 (50.0)	2 (50.0)	0
Total	352	351 (99.7)	62 (17.6)	312 (88.6)	161 (45.7)	144 (40.9)	264 (75.0)	324 (92.0)	334 (94.9)	295 (83.8)	333 (94.6)	330 (93.8)	238 (67.6)	26 (7.3)

• *E. coli* isolates from diarrheal and healthy weaning piglets and their environmental samples showed the low resistance rate to CT in 7.1% (13/182) from diarrheal pens and 7.6% (13/170) from healthy pens.

AMP: Ampicillin, AMC: Amoxicillin/clavulanic acid, CTX: Cefotaxime, CAZ: Ceftazidime, KAN: Kanamycin, STR: Streptomycin, GEN: Gentamicin, TET: Tetracycline, DO: Doxycycline, OTC: Oxytetracycline, ENR: Enrofloxacin, SXT: Sulfamethoxazole/trimethoprim, CT: Colistin



- Detection rates of *E. coli* isolates resistant to 13 tested antimicrobial agents were not different from both diarrheal and healthy piglet pens in this study.

Table 5.9 Detection rates of Class 1, 2 and 3 integrons of *Escherichia coli* from diarrheal and healthy weaning piglets and environmental samples in swine farm

Sampling source	Total no. of tested <i>E. coli</i> isolates	Total no. of integron positive isolates	No. (%) of <i>E. coli</i> isolate positive for		
			Class1	Class2	Class1 and 2
Diarrheal piglet pens	182	122 (67.0)	120 (98.4)	1 (0.8)	1 (0.8)
Rectal swab	130	93 (71.5) ^a	92 (98.9)	0	1 (1.1)
Feed from feeder	20	10 (50.0)	10 (100.0)	0	0
Water from nipple	12	5 (41.7)	4 (80.0)	1 (20.0)	0
Waste swab	16	11 (68.8)	11 (100.0)	0	0
Worker's hand	4	3 (75.0)	3 (100.0)	0	0
Healthy piglet pens	170	101 (59.4)	95 (94.0)	4 (4.0)	2 (2.0)
Rectal swab	116	64 (55.2) ^a	61 (95.3)	3 (4.7)	0
Feed from feeder	20	14 (70.0)	14 (100.0)	0	0
Water from nipple	9	8 (88.9)	8 (100.0)	0	0
Waste swab	21	13 (61.9)	10 (76.9)	1 (7.7)	2 (15.4)
Worker's hand	4	2 (50.0)	2 (100.0)	0	0
Total	352	223 (63.4)	215 (96.4) ^b	5 (2.2) ^b	3 (1.4)

- Class 1 integron of *E. coli* was more commonly detected in all tested samples in both diarrheal and healthy pens.

Diagrammatic representation of the procedure used for MLST

DNA EXTRACTION

PCR FOR 7
HOUSEKEEPING
GENES

DNA SEQUENCING

FORWARD AND
REWARD TRIMMING

MATCH ALLELE;
IN DATANASE

GENERATE CLONAL
COMPLEX &
PHYLYGENETIC TREE

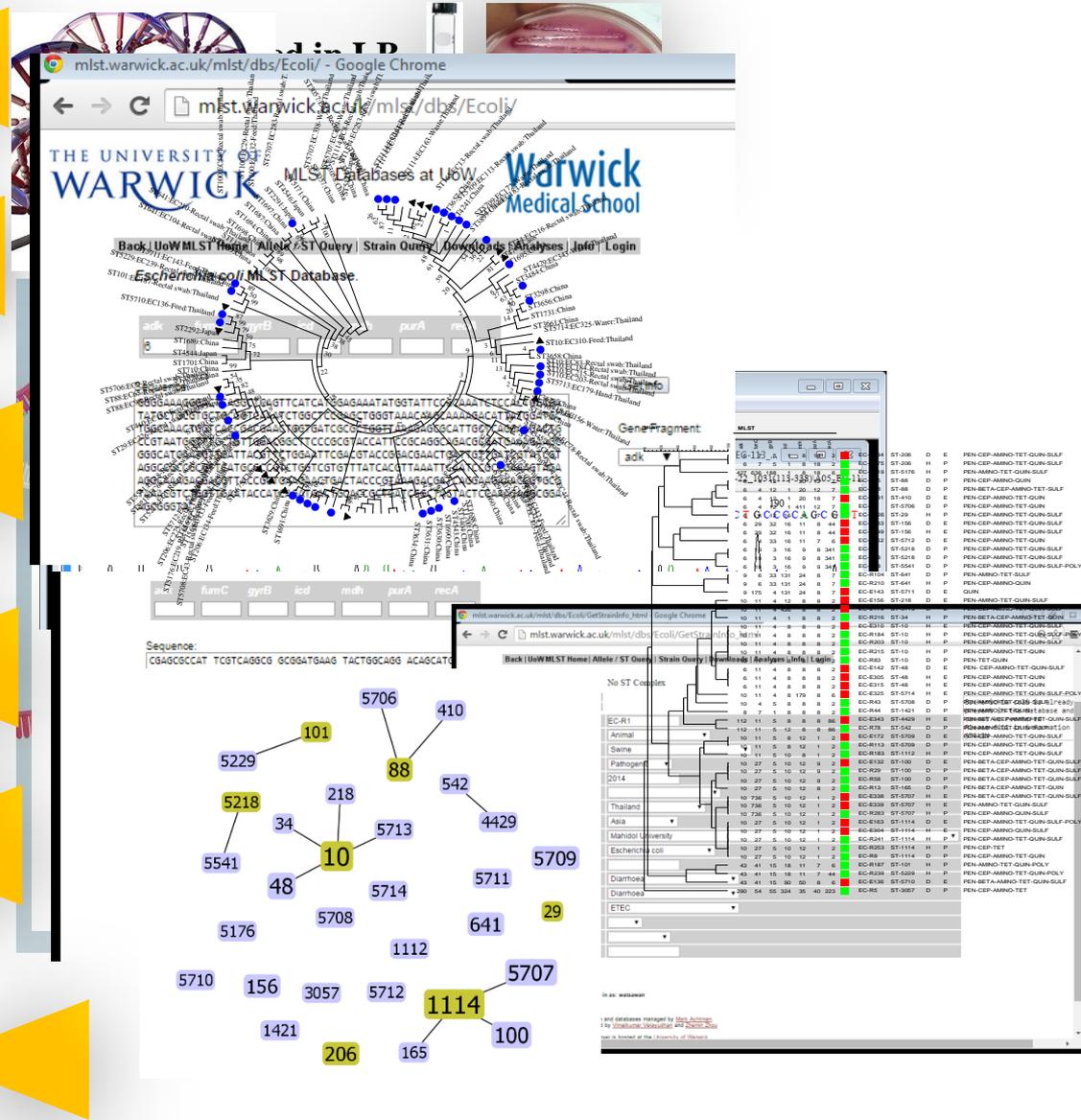
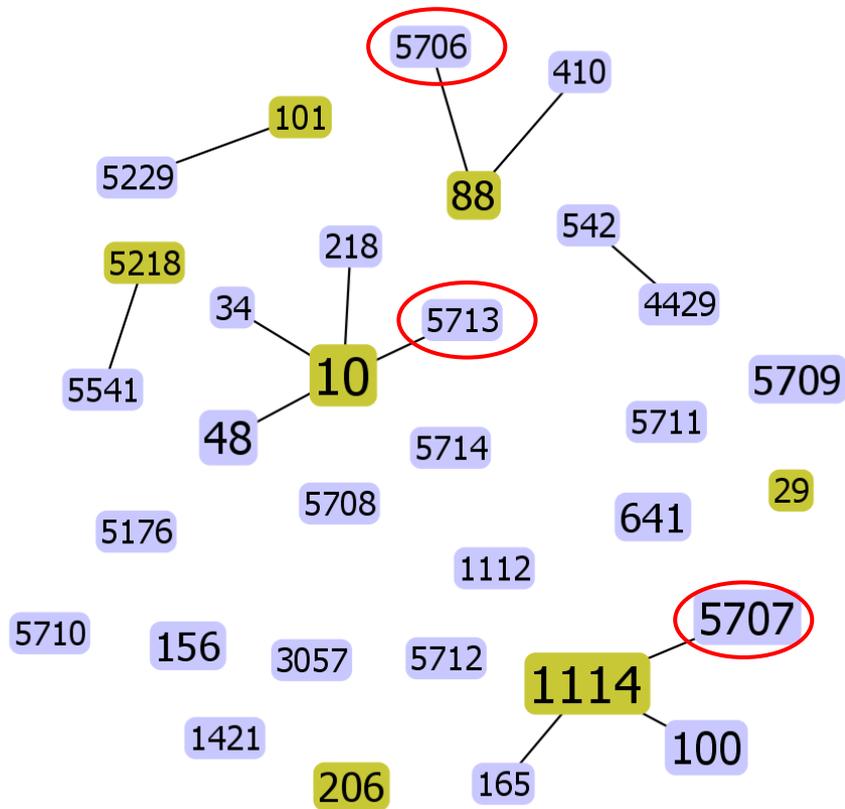


Table 5.14 Frequency of sequence types from 52 representative isolates from diarrheal and healthy weaning piglets and environmental samples

Sampling source	No. of isolate	No. of STs	Sequence types (STs)
Diarrheal piglet pens	29	26	
Rectal swab	17	14	ST10, ST88, ST100, ST165, ST542, ST641, ST1114, ST1421, ST3057, ST5218, ST5541, ST5706, ST5708, ST5709
Feed from feeder	7	7	ST48, ST100, ST156, ST206, ST410, ST5710, ST5711
Water from nipple	2	2	ST218, ST5712
Waste swab	2	2	ST1114, ST5709
Worker's hand	1	1	ST5713
Healthy piglet pens	23	18	
Rectal swab	14	11	ST10, ST29, ST34, ST101, ST206, ST641, ST1112, ST1114, ST5176, ST5229, ST5707
Feed from feeder	5	4	ST10, ST48, ST156, ST1114
Water from nipple	1	1	ST5714
Waste swab	3	2	ST4429, ST5707
Worker's hand	0	0	-

- The 52 *E. coli* isolates were assigned to 32 distinct STs with 11 new STs.
- Among 11 novel STs
 - 9 STs were detected from diarrheal piglet pens.
 - 2 STs were detected from healthy piglet pens.
- Majority of isolates were assigned to ST10 and ST1114.

Fig. 5.15 Clonal relationship of 32 STs among 52 *Escherichia coli* isolates identified by goeBURST. SLV are connected via black lines. The size of the circles is related to the number of isolates in STs.



- 18 out of 32 identified STs were grouped into 6 different CCs
- 14 STs were singleton

- Isolates from different sources were closely related especially **CC10 and CC114**.
- **CC10** were recovered **from rectal swab, feed, water, and hand samples** from both diarrheal and healthy piglet pens.
- **CC114** were also found in **rectal swab, feed, waste** samples from diarrheal and healthy piglet pens.

Table 5.16 Genetic variation among single locus variant members of novel sequence types (STs) and clonal complex of *Escherichia coli* isolates in this study

Novel ST	Closest match ST	Variant locus	Fragment size (bp)	Aligned position	Nucleotide	Genetic event
5713	10	<i>icd8 – icd426</i>	518	206	C → T	Single mutation ¹
5706	88	<i>mdh20 - mdh411</i>	452	334	G → C	Single mutation
5707	1114	<i>fumC27 - fumC736</i>	496	232	G → T	Single mutation

¹Mutation: The variant locus differing by a small number of nucleotides and were not observed elsewhere in the database as part of another ST

<p>S110:icd8 C G A C G C T G C A G T C G A G A A A G C C T A T A A A G C G G A G C G T A A A A T C T C C T S15713:icd426</p> <p>S110:icd8 T G G A A A T T T A C A C C G G T G A A A A T C C A C A C A G G T T T A T G G T C A G G A C S15713:icd426</p> <p>S110:icd8 T G G C T G C C T G C T G A A A C T C T T G A T C T G A T T C G T G A A A T A T C C G C T T G C S15713:icd426</p> <p>S110:icd8 T A A A G G T C C G C T G A C C A C T C C G G T T G G T G G C G G T A T T C G C T C T C T G A S15713:icd426</p> <p>S110:icd8 T T G C C T G C G C C A G G A A C T G G A T C T C T A C A T C T G C C T G C G T C C G G T A S15713:icd426</p> <p>S110:icd8 T A C T A T C A G G G C A C T C C A A G C C C G G T T A A A C A C C C T G A A C T G A C C G A S15713:icd426</p> <p>S110:icd8 G G T A T C T T C C G T G A A A A C T C G G A A G A C A T T T A T G C G G G T A T C G A A T S15713:icd426</p> <p>S110:icd8 A A G C A G A C T C T C C G A C C G C G A G A A A G T S A T T A A A T T C C T G C G T G A A S15713:icd426</p> <p>S110:icd8 A T G G G G G T G A A G A A A A T T C G C T T C C C G G A A C A T T G T G G T A T C G G T A T S15713:icd426</p> <p>S110:icd8 G C C G T G T T C G G A A G A A G G C A C C A A A C G T C T G G T T C G T G C A G C G A T C G A A T S15713:icd426</p> <p>S110:icd8 A C G C A A T T G C T A A C G A T C S15713:icd426</p>	<p>S188:mdh20 G G T G T A G C G C G T A A A C C G G G T A T G G A T C G T T C C G A C C T G T T T A A C G T T A A S15706:mdh411</p> <p>S188:mdh20 C G C C G G C A T C G T G A A A A A C C T G G T A C A G C A A G T T G C G A A A A C C T G C C C G A S15706:mdh411</p> <p>S188:mdh20 A A G C G T G C A T T G G T A T T A C T A A C C C G G T T A A C A C C A C A G T T G C G A T T S15706:mdh411</p> <p>S188:mdh20 G C T G C T G A A G T G C T G A A A A A G C C G G T G T T A T G A C A A A A C A A A C T G T T S15706:mdh411</p> <p>S188:mdh20 C G G C G T A C C A C G C T G G A T A T C A T T C G T T C C A A C A C T T T T S15706:mdh411</p> <p>S188:mdh20 T G A A A G C A A A C A G C C A G G C G A A G T T G A A G T G C C G G T T A T S15706:mdh411</p> <p>S188:mdh20 T C T G G T G T T A C C A T T C T G C C C T G C T G T C A C A G G T T T C C T G S15706:mdh411</p> <p>S188:mdh20 T A C C G A G C A G G A A G T G G C T G A T C T G A C C A A A G G T A T C C A G S15706:mdh411</p> <p>S188:mdh20 C T G A G G T G T T G A A G C G A A A G C C G G T G G C G G G T C T G C A A C S15706:mdh411</p> <p>S188:mdh20 G G S15706:mdh411</p>	<p>S1114:lumC27 C G A G C G C A T T C G T C A G G C G G C G G A T G A A G T A C T G G C A G G A C A G C A T G A C S15707:lumC736</p> <p>S1114:lumC27 G A C G A A T T C C C G C T G G C T A T C T G G C A G A C C G G C T C C G G C A C G C A A A G T A A S15707:lumC736</p> <p>S1114:lumC27 C A T G A A C A T G A A C G A A G T G C T G G C T A A C C G G C C A G T G A A T T A C T C G G C G S15707:lumC736</p> <p>S1114:lumC27 G T G T G C G G G A T G G A A C G T A A A G T T C A C C C T A A C G A C G A C G T G A A C A A A S15707:lumC736</p> <p>S1114:lumC27 A G C C A A A G T T C C A A C G A T G T C T T T C C G A C G G A T G A T G C A C G T T G C G G C T S15707:lumC736</p> <p>S1114:lumC27 G C T G G C G C T G C G C A A G C A A C T A T T C G T C A G C T T A A A A C C C T G A C A C A G A S15707:lumC736</p> <p>S1114:lumC27 C A C T G A A T G A G A A A T C C C G T G C T T T T G C C G A T A T C G T C A A A A T T G G T C G T S15707:lumC736</p> <p>S1114:lumC27 A C T C A C T T G C A G G A T G C C A C G C C G T T A A C G C T G G G G C A G G A G A T T T C C G G S15707:lumC736</p> <p>S1114:lumC27 C T G G A T A G C G A T G C T C G A G C A T A A T C T C A A A C A T A T C G A A T A C A G C T G C S15707:lumC736</p> <p>S1114:lumC27 C T C A C G T A G C G G A A C T G G C S15707:lumC736</p>
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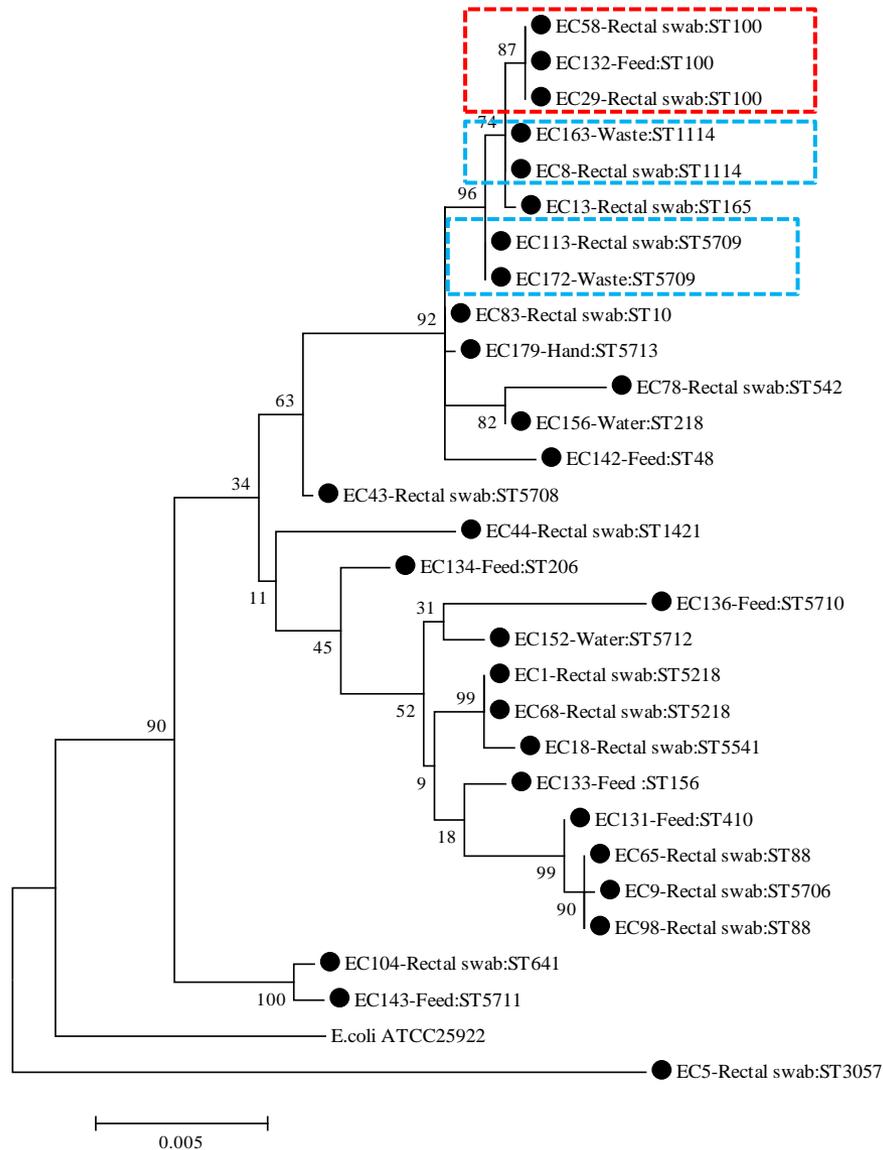


Fig. 5.21

Intra-relationship analysis

- 29 *E. coli* isolates from diarrheal weaning piglets and environmental samples were analyzed by ML tree based on the concatenated sequences of seven housekeeping genes loci (3,423 bp).
- Same STs of *E. coli* isolates in diarrheal piglet pens were isolated from different sources.
- ST100 represented isolates recovered from rectal swab and feed samples.
- ST1114 and ST5709 represented isolates recovered from rectal and waste swab samples.
- These results indicated that *E. coli* isolates from rectal swab and environmental samples might be originated from the same clone.

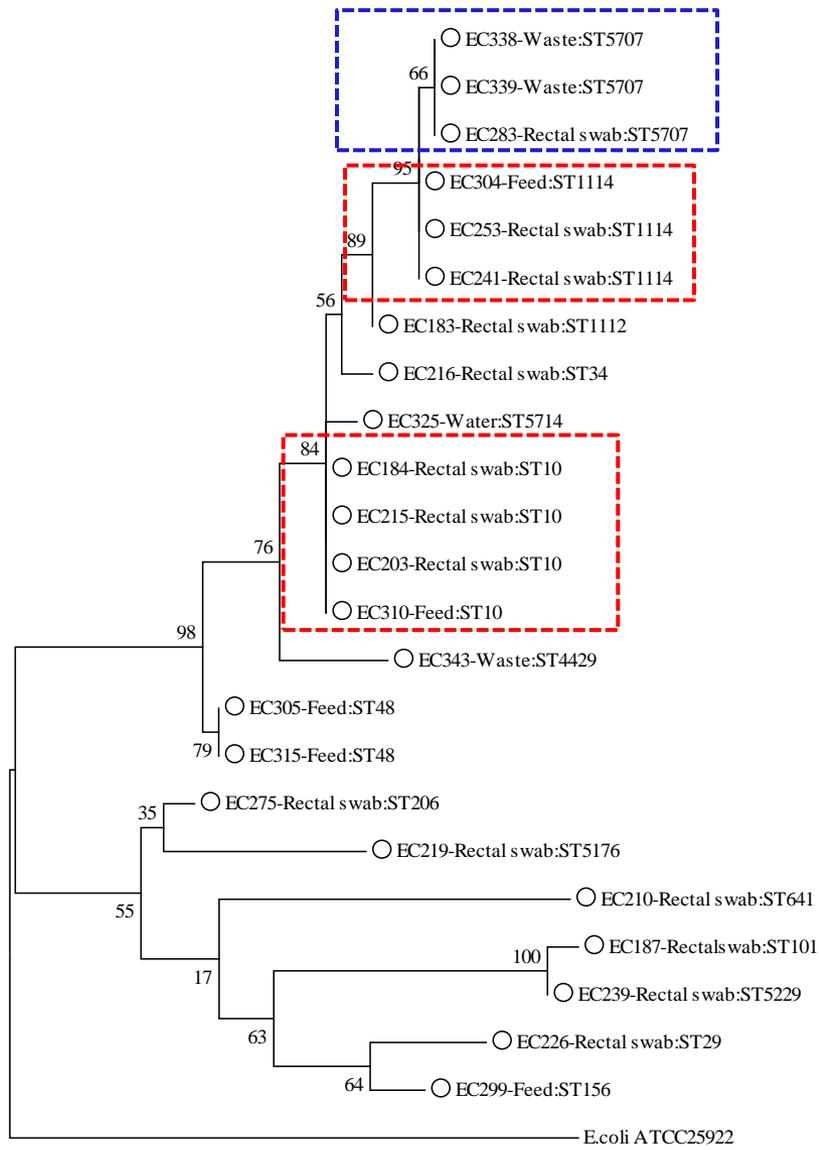


Fig. 5.22

Intra-relationship analysis

- 23 *E. coli* isolates from weaning piglets without diarrhea and environmental samples were also analyzed by ML
- The same STs were isolated from different sources.
- ST10 and ST1114 represented isolates obtained from rectal swab and feed samples.
- ST5707 represented isolates obtained from rectal and waste swab samples.
- These results indicated that *E. coli* from rectal swab and environmental samples might be originated from same origin

Inter-relationship analysis

- 52 *E. coli* isolates from weaning piglets with and without diarrheal were analyzed phylogenetic relationship by using ML.
- ST10, ST48, ST156, ST206, ST641 and ST1114 were isolated from both diarrheal and healthy piglet pens.

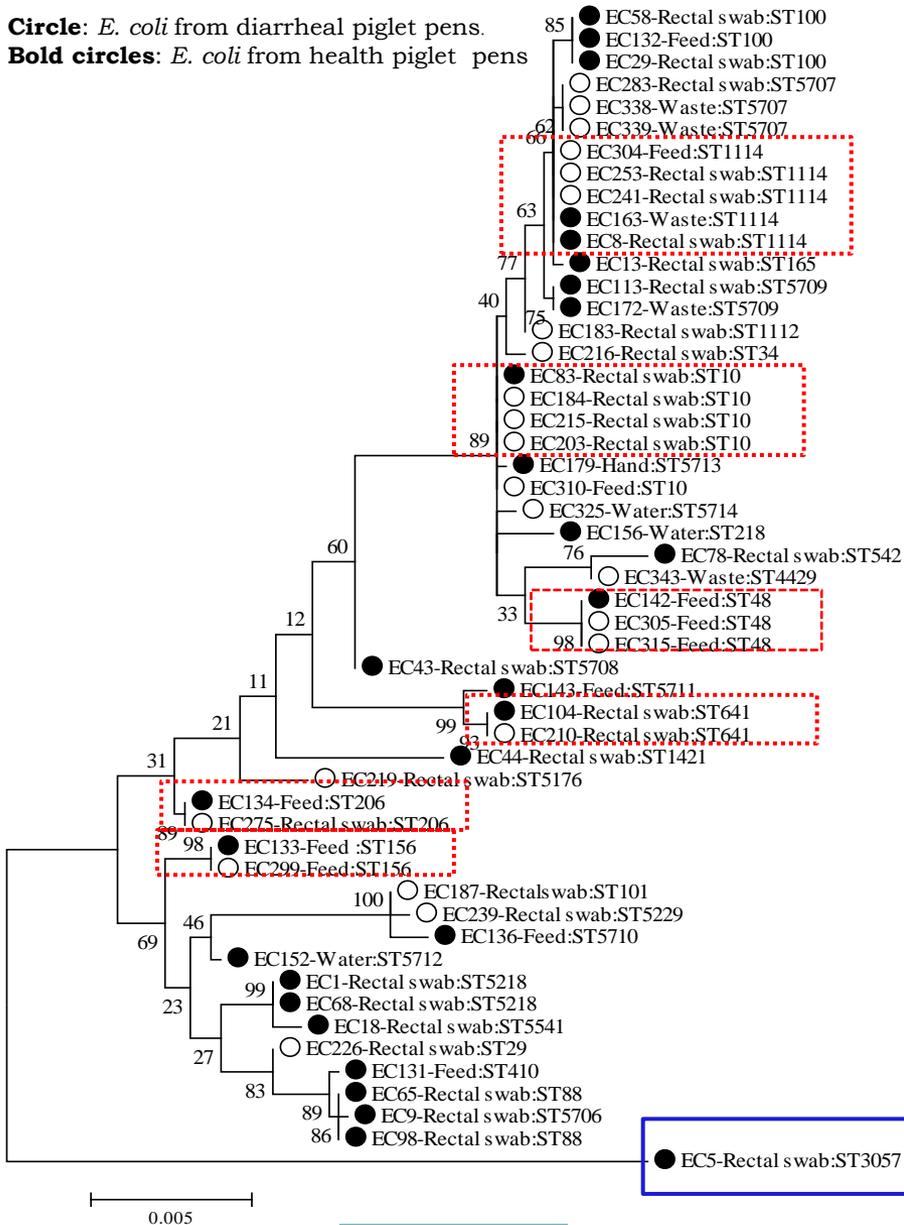
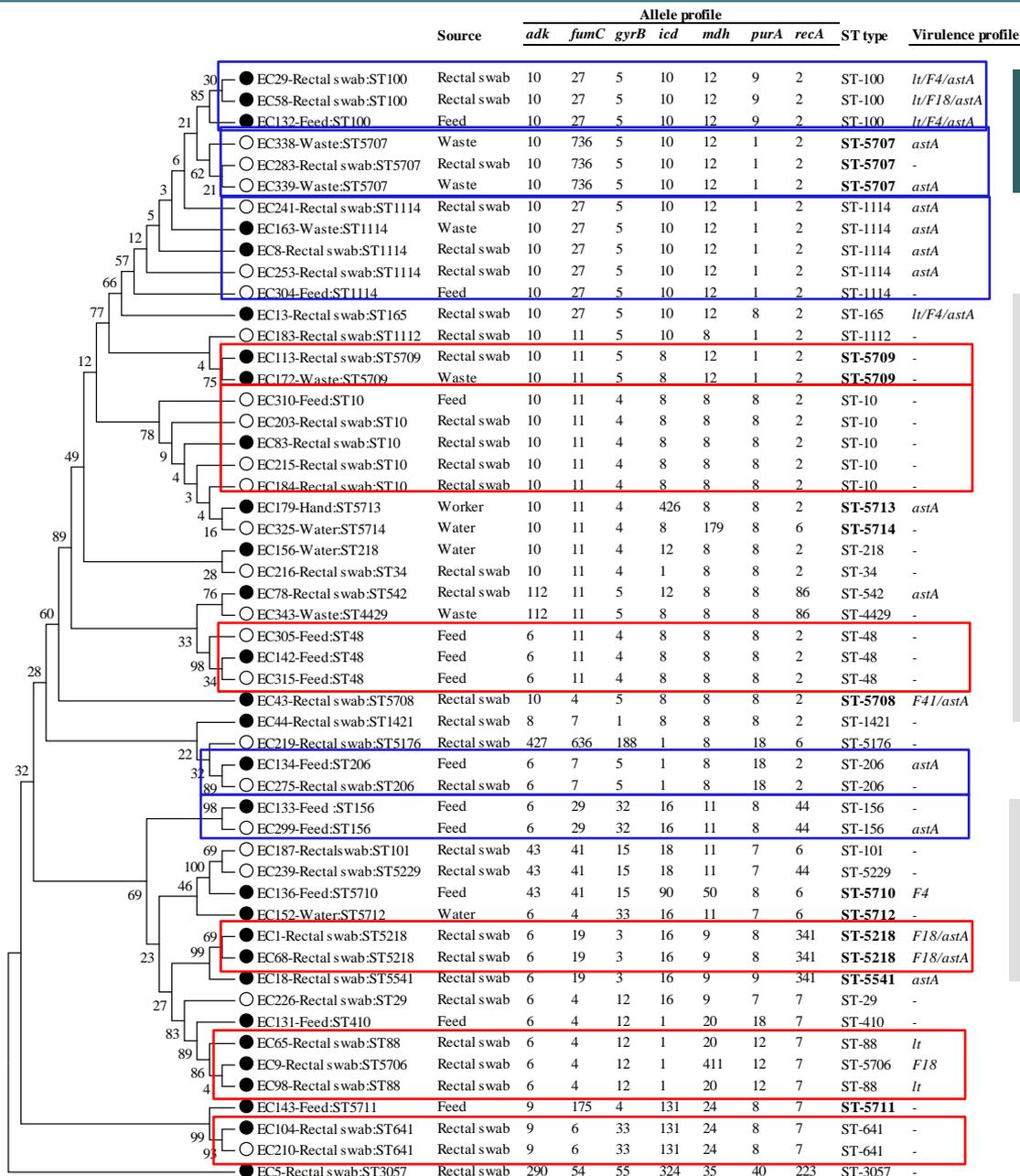


Fig. 5.23

- ST3057 isolated from rectal swab sample in diarrheal piglet pen was demonstrated different from other isolates that presenting high degree of genetic diversity in ML



Association between ST and virulence profiles

- Association between ST and virulence profiles were observed in 6 STs, ST10, ST48, ST88, ST641, ST5709 and ST5218.
- 4 STs were non pathogenic *E. coli*.
- ST88 (*lt*)
- ST5218 (*F18*⁺/*astA*⁺)
- 5 STs; ST100, ST5707, ST1114, ST206, and ST156 showed different virulence profiles within same STs.

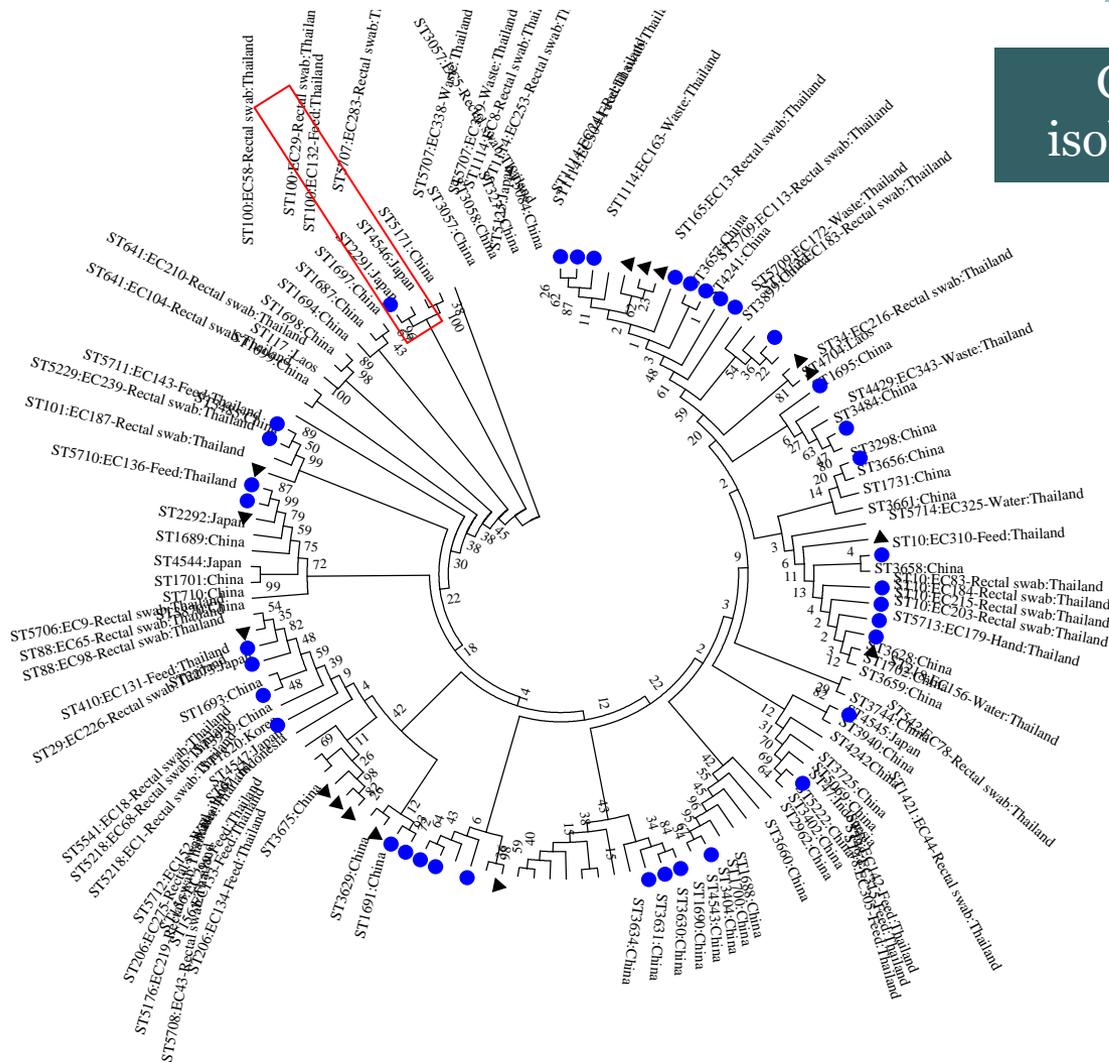
Fig. 5.24 The association between ST and virulence profiles of 52 *E. coli* isolates

Association between ST and antimicrobial resistance patterns

- Most of STs from MLST analysis and profiles of antimicrobial resistance phenotypes and integrons were not correlated.
- The same STs showed highly variable patterns of multi-antimicrobial resistance including ST100, ST5707, ST1114, ST10, ST48, ST5218, ST88, and ST641.
- 3 STs including ST156, ST206, and ST5709 revealed correlation between STs and antimicrobial resistance profiles.
- Interestingly, ST5709 were detected from rectal swab and waste swab samples in diarrheal piglet pens that showed correlation with virulence and antimicrobial resistance profiles

Source	ST type	ABO resistance profile	Integron
● EC29-Rectal swab:ST100	Rectal swab	ST-100 AMP-AMC-CTX-CAZ-KAN-TET-DO-OTC-ENR-SXT	Int1
● EC58-Rectal swab:ST100	Rectal swab	ST-100 AMP-AMC-CTX-CAZ-KAN-STR-GEN-TET-DO-OTC-ENR-SXT	Int1
● EC132-Feed:ST100	Feed	ST-100 AMP-AMC-CTX-CAZ-KAN-GEN-TET-DO-OTC-ENR-SXT	-
○ EC338-Waste:ST5707	Waste	ST-5707 AMP-AMC-CTX-CAZ-KAN-STR-GEN-TET-DO-OTC-ENR-SXT	Int1- Int2
○ EC283-Rectal swab:ST5707	Rectal swab	ST-5707 AMP-CTX-CAZ-KAN-GEN-ENR-SXT	Int2
○ EC339-Waste:ST5707	Waste	ST-5707 AMP-STR-GEN-TET-DO-OTC-ENR-SXT	Int2
○ EC241-Rectal swab:ST1114	Rectal swab	ST-1114 AMP-CTX-CAZ-KAN-STR-GEN-TET-DO-OTC-ENR-SXT	Int1
● EC163-Waste:ST1114	Waste	ST-1114 AMP-CTX-CAZ-STR-GEN-TET-DO-OTC-ENR-SXT-CT	-
● EC8-Rectal swab:ST1114	Rectal swab	ST-1114 AMP-CTX-KAN-GEN-TET-DO-OTC-ENR	-
○ EC253-Rectal swab:ST1114	Rectal swab	ST-1114 AMP-CTX-TET-DO-OTC	-
○ EC304-Feed:ST1114	Feed	ST-1114 AMP-CTX-CAZ-KAN-STR-GEN-ENR-SXT	Int1
● EC13-Rectal swab:ST165	Rectal swab	ST-165 AMP-AMC-CTX-CAZ-KAN-GEN-TET-DO-OTC-ENR	Int1
○ EC183-Rectal swab:ST1112	Rectal swab	ST-1112 AMP-CTX-STR-GEN-TET-DO-OTC-ENR-SXT	Int1
● EC113-Rectal swab:ST5709	Rectal swab	ST-5709 AMP-CTX-STR-GEN-TET-DO-OTC-ENR-SXT	-
● EC172-Waste:ST5709	Waste	ST-5709 AMP-CTX-STR-GEN-TET-DO-OTC-ENR-SXT	Int1
○ EC310-Feed:ST10	Feed	ST-10 AMP-CTX-KAN-STR-GEN-TET-DO-OTC-ENR-SXT	Int1
○ EC203-Rectal swab:ST10	Rectal swab	ST-10 AMP-CTX-KAN-STR-GEN-TET-DO-OTC-ENR-SXT	Int1
● EC83-Rectal swab:ST10	Rectal swab	ST-10 AMP-TET-DO-OTC-ENR	Int1
○ EC215-Rectal swab:ST10	Rectal swab	ST-10 AMP-CTX-GEN-TET-DO-OTC-ENR	-
○ EC184-Rectal swab:ST10	Rectal swab	ST-10 AMP-CTX-STR-GEN-TET-DO-OTC-ENR-SXT-CT	Int1
● EC179-Hand:ST5713	Worker	ST-5713 AMP-CTX-STR-GEN-TET-DO-OTC-ENR-SXT	Int1
○ EC325-Water:ST5714	Water	ST-5714 AMP-CTX-CAZ-STR-GEN-TET-DO-OTC-ENR-SXT-CT	Int1
● EC156-Water:ST218	Water	ST-218 AMP-STR-TET-DO-OTC-ENR-SXT	Int2
○ EC216-Rectal swab:ST34	Rectal swab	ST-34 AMP-AMC-CTX-CAZ-STR-GEN-TET-DO-OTC-ENR	-
○ EC78-Rectal swab:ST542	Rectal swab	ST-542 AMP-STR-TET-DO-OTC-ENR-SXT	Int1-Int2
○ EC343-Waste:ST4429	Waste	ST-4429 AMP-AMC-CTX-CAZ-KAN-STR-GEN-TET-DO-OTC-ENR-SXT	Int1- Int2
○ EC305-Feed:ST48	Feed	ST-48 AMP-CTX-CAZ-KAN-STR-GEN-TET-DO-OTC-ENR	-
● EC142-Feed:ST48	Feed	ST-48 AMP-CTX-KAN-STR-GEN-TET-DO-OTC-ENR-SXT	Int1
○ EC315-Feed:ST48	Feed	ST-48 AMP-CTX-CAZ-STR-GEN-TET-DO-OTC-ENR	Int1
● EC43-Rectal swab:ST5708	Rectal swab	ST-5708 AMP-KAN-STR-GEN-TET-DO-OTC-ENR-SXT	Int1
● EC44-Rectal swab:ST1421	Rectal swab	ST-1421 AMP-KAN-GEN-TET-DO-OTC-ENR	-
○ EC219-Rectal swab:ST5176	Rectal swab	ST-5176 AMP-KAN-GEN-TET-DO-OTC-ENR-SXT	Int1
● EC134-Feed:ST206	Feed	ST-206 AMP-CTX-CAZ-KAN-STR-GEN-TET-DO-OTC-ENR-SXT	Int1
○ EC275-Rectal swab:ST206	Rectal swab	ST-206 AMP-CTX-CAZ-KAN-STR-GEN-TET-DO-OTC-ENR-SXT	Int1
● EC133-Feed:ST156	Feed	ST-156 AMP-CTX-KAN-STR-GEN-TET-DO-OTC-ENR-SXT	Int1
○ EC299-Feed:ST156	Feed	ST-156 AMP-CTX-KAN-STR-GEN-TET-DO-OTC-ENR-SXT	-
○ EC187-Rectalswab:ST101	Rectal swab	ST-101 AMP-STR-TET-DO-OTC-ENR-CT	-
○ EC239-Rectal swab:ST5229	Rectal swab	ST-5229 AMP-CTX-CAZ-GEN-TET-DO-OTC-ENR-CT	-
● EC136-Feed:ST5710	Feed	ST-5710 AMP-AMC-STR-GEN-TET-DO-OTC-ENR-SXT	Int1
● EC152-Water:ST5712	Water	ST-5712 AMP-CTX-KAN-GEN-TET-DO-OTC-ENR	-
● EC1-Rectal swab:ST5218	Rectal swab	ST-5218 AMP-CTX-CAZ-STR-GEN-TET-OTC-ENR-SXT	Int1
● EC68-Rectal swab:ST5218	Rectal swab	ST-5218 AMP-CTX-CAZ-KAN-STR-GEN-TET-DO-OTC-ENR-SXT	-
● EC18-Rectal swab:ST5541	Rectal swab	ST-5541 AMP-CTX-STR-GEN-TET-DO-OTC-ENR-SXT	Int1
○ EC226-Rectal swab:ST29	Rectal swab	ST-29 AMP-CTX-STR-GEN-TET-DO-OTC-ENR-SXT	Int1
○ EC131-Feed:ST410	Feed	ST-410 AMP-CTX-STR-GEN-TET-DO-OTC-ENR	-
● EC65-Rectal swab:ST88	Rectal swab	ST-88 AMP-CTX-KAN-ENR	-
● EC9-Rectal swab:ST5706	Rectal swab	ST-5706 AMP-CTX-CAZ-KAN-STR-GEN-TET-OTC-ENR	-
● EC98-Rectal swab:ST88	Rectal swab	ST-88 AMP-AMC-CTX-CAZ-STR-GEN-TET-DO-OTC-SXT	Int1
● EC143-Feed:ST5711	Feed	ST-5711 ENR	-
● EC104-Rectal swab:ST641	Rectal swab	ST-641 AMP-STR-GEN-TET-DO-OTC-SXT	Int1
○ EC210-Rectal swab:ST641	Rectal swab	ST-641 AMP-CTX-CAZ-STR-GEN-ENR	-
● EC5-Rectal swab:ST3057	Rectal swab	ST-3057 AMP-CTX-CAZ-GEN-TET-OTC	-

Fig. 5.25 The correlation between MLST and virulence profiles of 52 *E. coli* isolates



Genetic relationship of *E. coli* isolated from swine sources in Asia

- The result demonstrated that the 32 STs of this study showed diverse genetic background.
- ST3057 that showed high genetic diversity for this study but it was identical with *E. coli* isolates from China.

Fig. 5.26

Genetic relationship of *Escherichia coli* isolates. The ML tree was constructed based on the concatenated sequences of seven housekeeping genes loci (3,423 bp) of 95 STs from 115 isolates (32 STs from 52 isolates from this study) obtained from swine sources in Asia from database.

Conclusion and Recommendation

- 52 *E. coli* isolates were generated 32 distinct STs with 11 new STs from indicating that they were genetically diverse.
- ST10 and ST1114 were the common ST circulating in both diarrheal and healthy weaning piglets and their environments.
- Phylogenetic analysis revealed that the same STs in various sources among diarrheal and healthy piglet pens were found indicating the same clone contaminated in these sources causing the infection.

Conclusion and Recommendation

- ST10, ST48, ST156, ST206, ST641 and ST1114 were isolated from both diarrheal and healthy piglet pens.
- Notably, it was demonstrated that *E. coli* from either diarrheal or healthy weaning piglets could be a source of transmission through their environments, or vice versa.
- Moreover, the STs of *E. coli* isolates and virulence/ antimicrobial resistance/integrans were not correlated in this study.

THANK YOU FOR ATTENTION

