



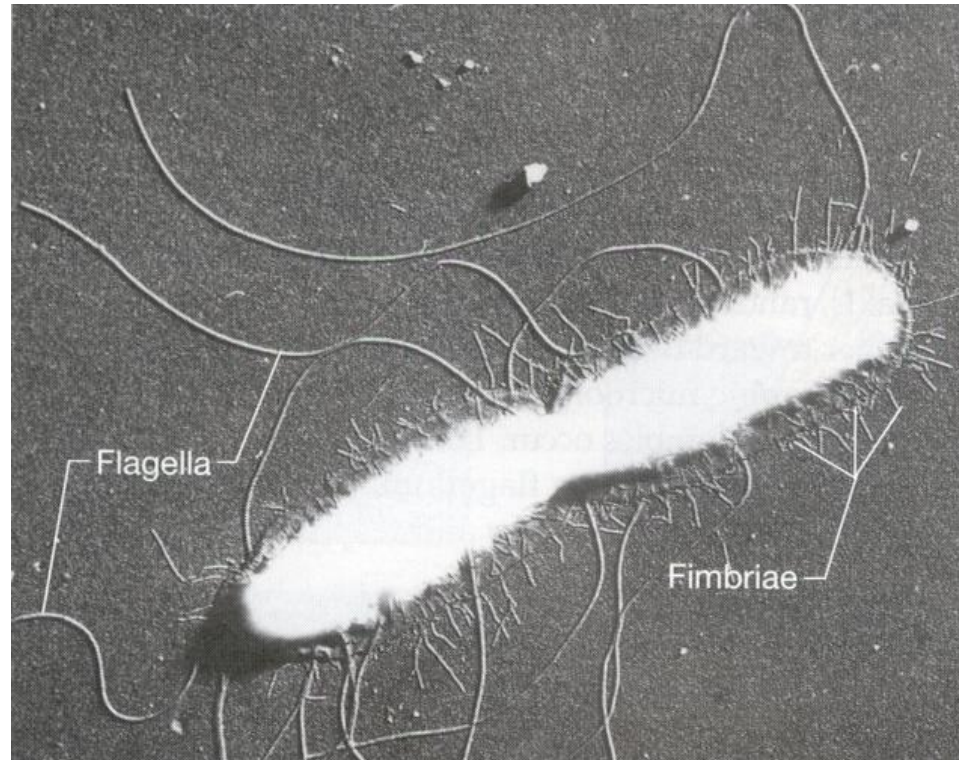
How NSSLRL (Ireland) has implemented WGS-based AMR Surveillance

Niall De Lappe

Galway Microbiology Reference
Laboratory Services

National *Salmonella*, *Shigella* & *Listeria* Reference Laboratory

National Carbapenemase Producing Enterobacterales Reference Laboratory (+ COVID)

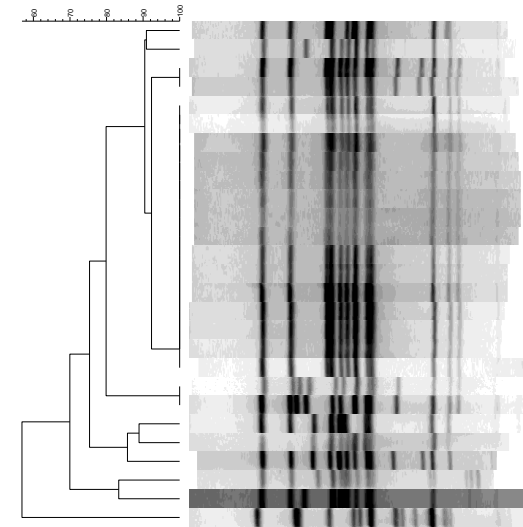
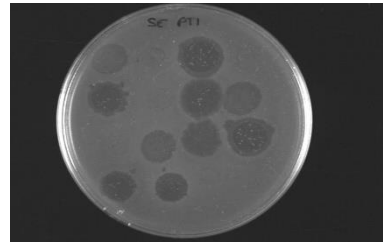




Techniques



- Serotyping
- Phage Typing
- PFGE
- MLVA
- AST



– Disk Diffusion

– Broth Microdilution with TREK Sensititre

2010

– EUCAST Guidelines

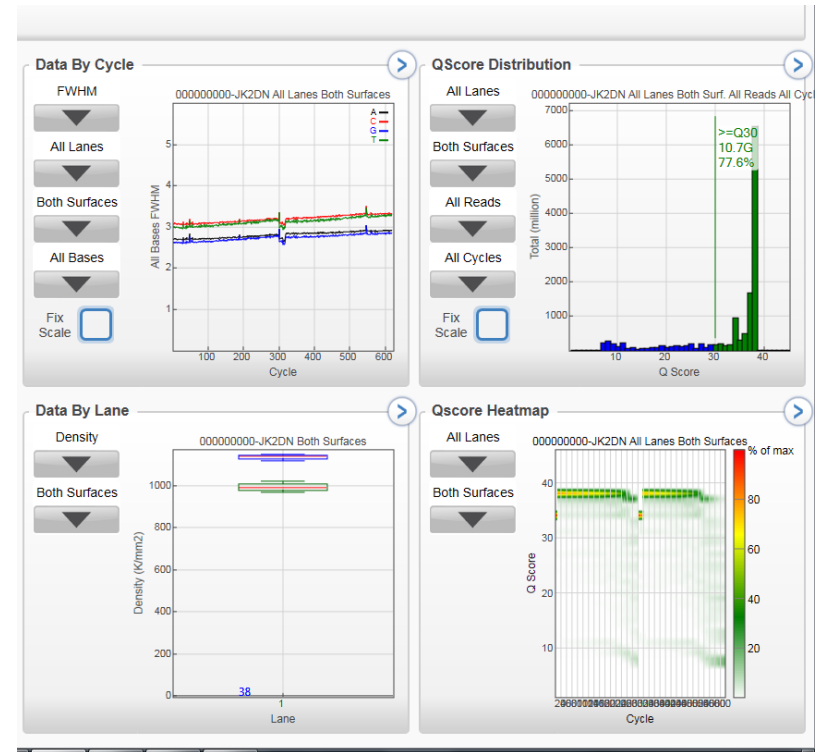


WGS The Beginning

- End of 2015
- Extracted DNA and sent to PHE
- FileZilla software
- Downloaded fastq files
- Analysed

2018

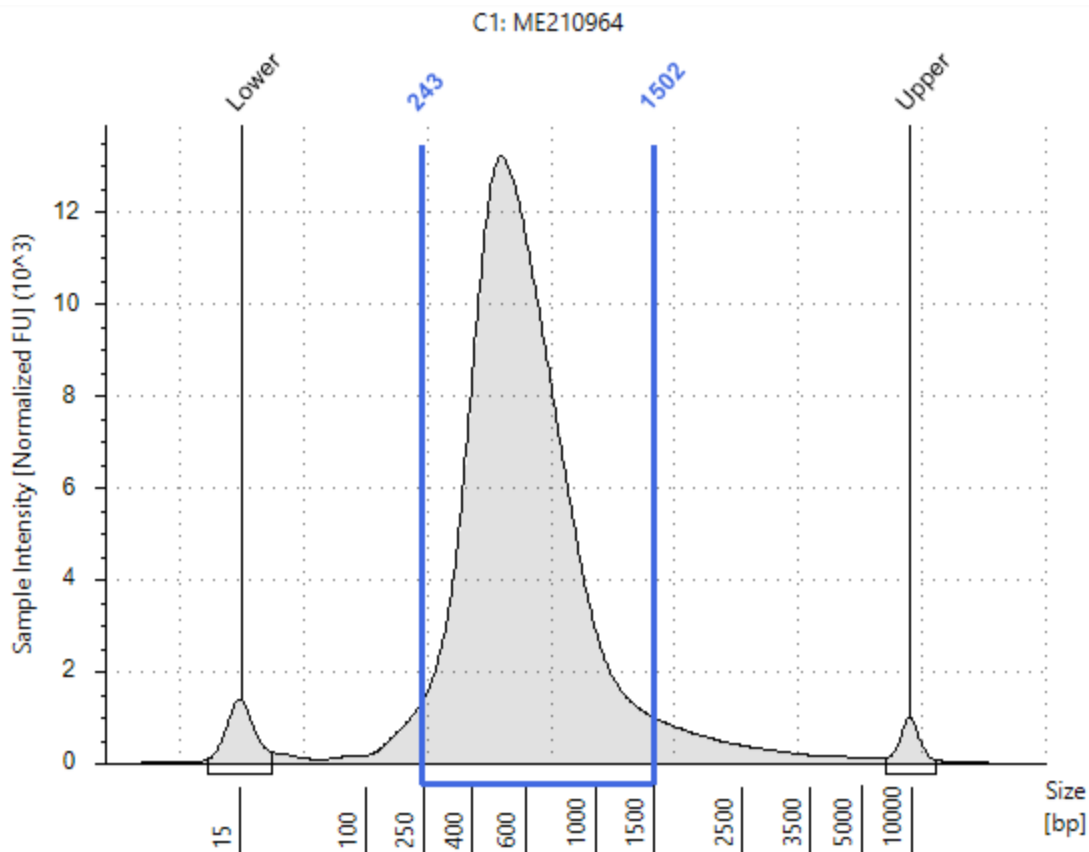
- Qiagen EZ1
- MiSeq
- Illumina Nextera XT
 - Changed to Flex/DNA Prep (July 2019)
- MiSeq V3 kit
 - 300bp reads



Robotic Liquid Handler



Tapestation



Analysis

- BioNumerics software
 - BioNumerics
 - EnteroBase
 - CGE
 - pubMLST
 - PathogenWatch



- Ran WGS and Conventional Techniques in Tandem until October 2018

AST Contamination

Broth MIC		Resistance Genes
ASSuTTmNaCp	v	none
ASuTNaCpAzt	v	ASuT
T	v	none (<i>E.coli</i>)
ASu	v	none

On repeat all matched with resistance genes

ASuTTmNaCp

Test Name	Specimen /	Iso.	Organism	Source	Species
MS180241					
MS180241		1	Salmonella species	Faeces	Human

Plates Read
 Show All
 EUVSEC
 EUVSEC

Vizion Review Stored Image - EUVSEC, Sec. 1 (MS180241, 1)

	1	2	3	4	5	6	7	8	9	10	11	12
A	SMX 1024	TMP 32	CIP 8	TET 64	MERO 16	AZI 64	NAL 128	CHL 128	TIGECY 8	COL 16	AMP 64	GEN 32
B	SMX 512	TMP 16	CIP 4	TET 32	MERO 8	AZI 32	NAL 64	CHL 64	TIGECY 4	COL 8	AMP 32	GEN 16
C	SMX 256	TMP 8	CIP 2	TET 16	MERO 4	AZI 16	NAL 32	CHL 32	TIGECY 2	COL 4	AMP 16	GEN 8
D	SMX 128	TMP 4	CIP 1	TET 8	MERO 2	AZI 8	NAL 16	CHL 16	TIGECY 1	COL 2	AMP 8	GEN 4
E	SMX 64	TMP 2	CIP 0.5	TET 4	MERO 1	AZI 4	NAL 8	CHL 8	TIGECY 0.5	COL 1	AMP 4	GEN 2
F	SMX 32	TMP 1	CIP 0.25	TET 2	MERO 0.5	AZI 2	NAL 4	FOT 1	TIGECY 0.25	TAZ 2	AMP 2	GEN 1
G	SMX 16	TMP 0.5	CIP 0.12	CIP 0.03	MERO 0.25	MERO 0.06	FOT 4	FOT 0.5	TAZ 8	TAZ 1	AMP 1	GEN 0.5
H	SMX 8	TMP 0.25	CIP 0.06	CIP 0.015	MERO 0.12	MERO 0.03	FOT 2	FOT 0.25	TAZ 4	TAZ 0.5	POS	POS

Interpretation Key

- Susceptible
- Intermediate
- Resistant
- No Interpretation



SIR

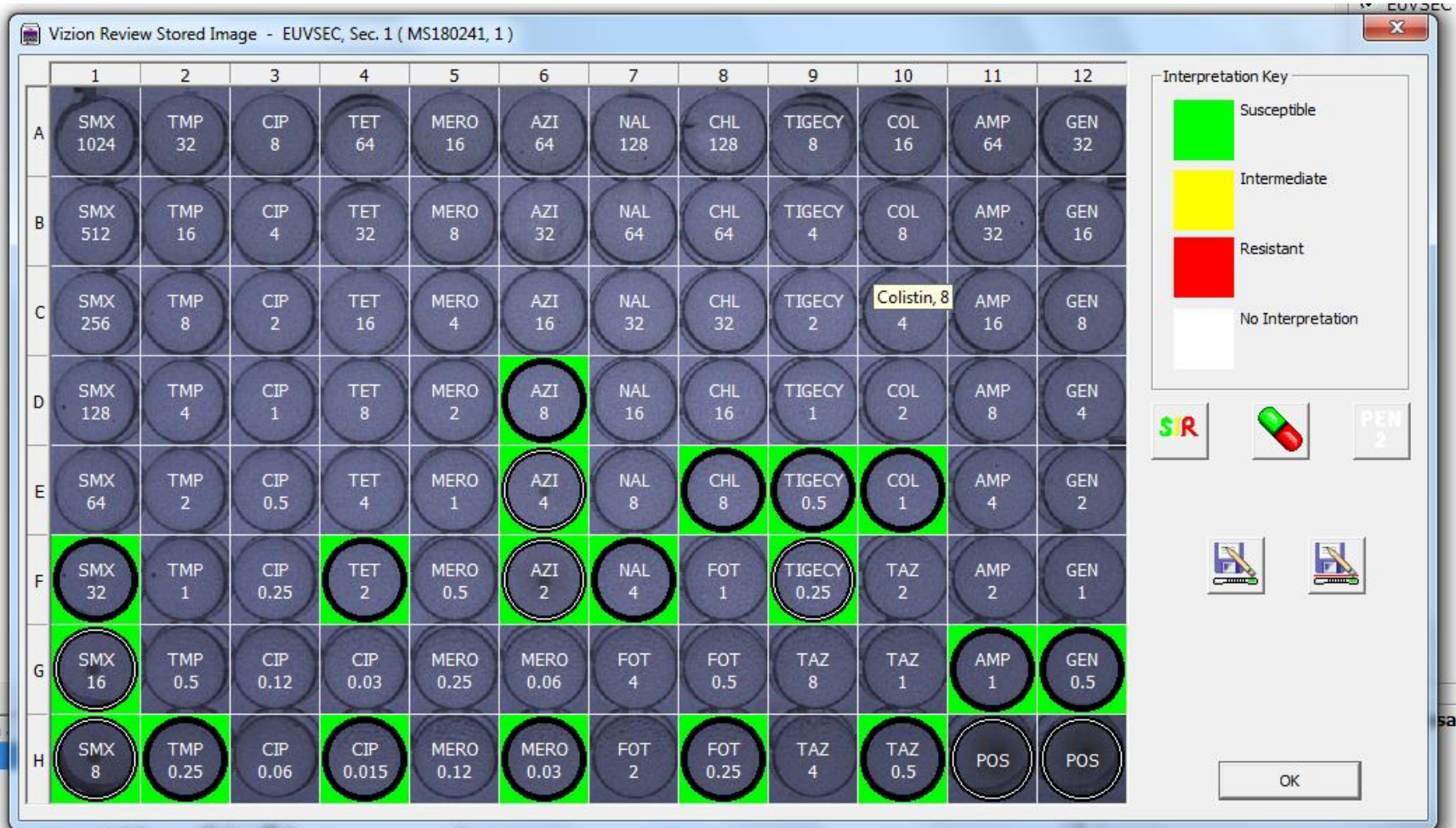




PEN 2

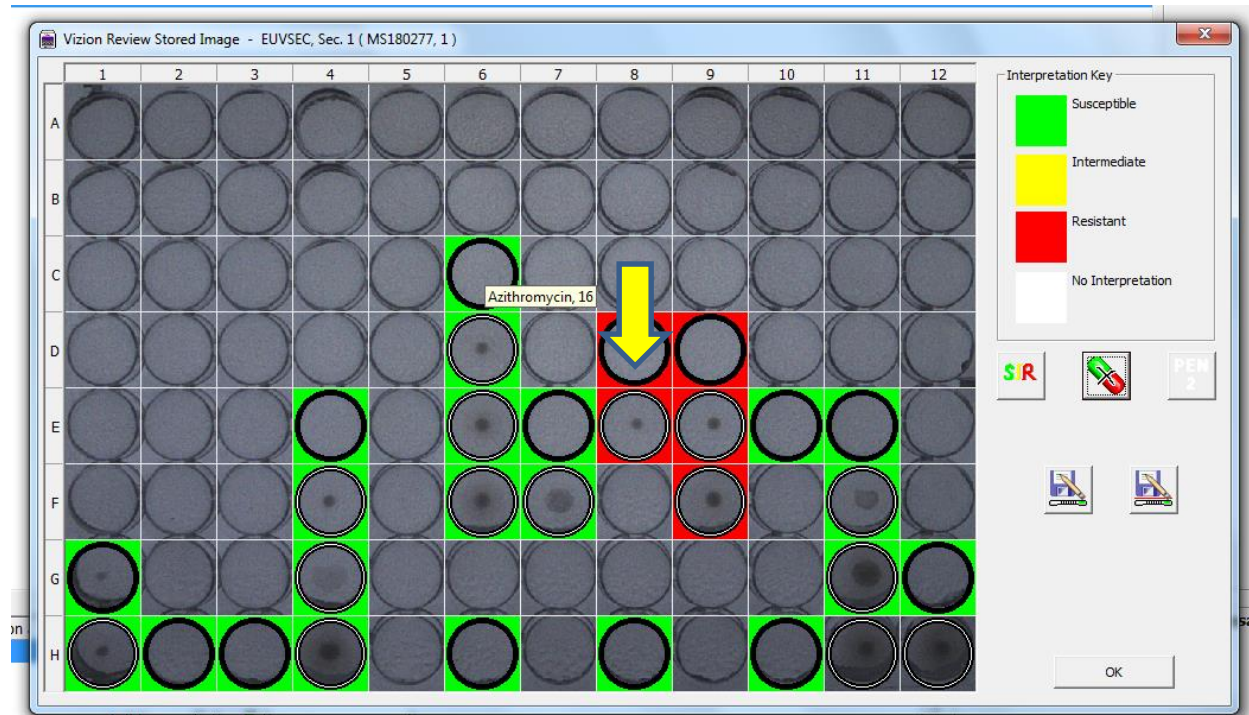



No Resistance



Validation

- 412/418 isolates correlated (98.6%)
- Resistance to Chloramphenicol (n=3) with no corresponding gene
- MIC=16



Discrepancies

- Azithromycin resistance (n=2) with no corresponding genes
 - MIC=32 & MIC=64
 - ? Variations in Efflux Pumps
 - *acrB* efflux pump
- *su1* gene present & MIC=16 (Susceptible)

Trimethoprim *Salmonella*

• No <i>dfrA</i> genes		MIC ≤2	
• <i>dfrA1</i>	100%	n=8	>32
• <i>dfrA5</i>	100%	n=1	>32
• <i>dfrA7</i>	100%	n=6	>32
• <i>dfrA12</i>	100%	n=14	>32
• <i>dfrA14</i>	99.59-.79	n=12	>32

1. Azithromycin has been used in the treatment of enteric infections, primarily with *Salmonella* Typhi and *Shigella* spp. For wild-type isolates of both species, the MICs are ≤ 16 mg/L and the inhibition zone diameters for the azithromycin 15 μ g disk ≥ 12 mm.

Salmonella

Resistant

mph(A) n= 4 MIC = 64

No genes n=35 MIC = 32

? Efflux pumps

ST65 *S.*Brandenburg

Susceptible

No genes n=28 MIC = 16

No genes n=619 MIC ≤ 8

ESBL in *Salmonella*

- ESBL Phenotype
 - blaCTX-M-15 (2)
 - blaCTX-M-14 (1)
 - blaCTX-M-14b (1)
 - blaSHV-12 (2)
 - blaCMY-2 (1)
 - blaDHA-1 (1)

Non-ESBL Phenotype

- *bla*TEM
 - -1A (2), 1B (186), -1C (3), -57 (2), -104 (1), -126 (38), -143 (1), -166 (1)
 - *bla*CARB-2 (23)
 - *bla*OXA-1 (3), *bla*OXA-10 (1)
- bla*TEM-126 (99.77 & 99.88%)
- bla*TEM-143 (95.55%)

NCBI Reference Gene Catalog

TEM-126



Search

db version: 2021-03-01.1 [Changelog](#)

Bacterial Antimicrobial Resistance Reference Gene Database

Filters

Page 1 of 1 Records per Page 20 Choose columns Download

Displaying 1 - 1 of 1

#	allele	gene fa...	product name	scope	type	subtype	class	subclass	refseq pr...	refseq n...	genbank...	genbank...	curated ...
1	blaTEM-...	blaTEM	class A extended-spectrum beta-lactamase TEM-126	core	AMR	AMR	BETA-LA...	CEPHALOSPORIN	WP_063...	NG_050...	AAT4574...	AY62819...	No

blaTEM-126

Nucleotide Differences

Some with 2 (99.77% ID) and some with 1 (99.88%) nt differences.

Both resulted in the same 1 Amino Acid Substitution

Alignments Result Summary Guide Tree Phylogenetic Tree Results View
Download Alignment File

CLUSTAL O(1.2.4) multiple sequence alignment

```
blaTEM_126_aa      MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRP 60
MS170170_TEM      MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRP 60
MS180027_TEM      MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRP 60
MS190261_TEM      MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRP 60
MS210270_TEM      MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRP 60
MS210416_TEM      MSIQHFRVALIPFFAAFCLPVFAHPETLVKVKDAEDQLGARVGYIELDLNSGKILESFRP 60
*****

blaTEM_126_aa      EERFPMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVREL 120
MS170170_TEM      EERFPMISTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVREL 120
MS180027_TEM      EERFPMISTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVREL 120
MS190261_TEM      EERFPMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVREL 120
MS210270_TEM      EERFPMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVREL 120
MS210416_TEM      EERFPMSTFKVLLCGAVLSRVDAGQEQLGRRIHYSQNDLVEYSPVTEKHLTDGMTVREL 120
*****

blaTEM_126_aa      CSAAITMSDNTAANLLLTIGGPKELTAF LHNMGDHSVTRLDRWEPELNEAIPNDERDRTT 180
MS170170_TEM      CSAAITMSDNTAANLLLTIGGPKELTAF LHNMGDHSVTRLDRWEPELNEAIPNDERDRTT 180
MS180027_TEM      CSAAITMSDNTAANLLLTIGGPKELTAF LHNMGDHSVTRLDRWEPELNEAIPNDERDRTT 180
MS190261_TEM      CSAAITMSDNTAANLLLTIGGPKELTAF LHNMGDHSVTRLDRWEPELNEAIPNDERDRTT 180
MS210270_TEM      CSAAITMSDNTAANLLLTIGGPKELTAF LHNMGDHSVTRLDRWEPELNEAIPNDERDRTT 180
MS210416_TEM      CSAAITMSDNTAANLLLTIGGPKELTAF LHNMGDHSVTRLDRWEPELNEAIPNDERDRTT 180
*****

blaTEM_126_aa      PAAMATTLRKLLTGELLTASRQQLIDWMEADKVAGPLLR S ALPAGWFIADKSGAGERG S 240
MS170170_TEM      PAAMATTLRKLLTGELLTASRQQLIDWMEADKVAGPLLR S ALPAGWFIADKSGAGERG S 240
MS180027_TEM      PAAMATTLRKLLTGELLTASRQQLIDWMEADKVAGPLLR S ALPAGWFIADKSGAGERG S 240
MS190261_TEM      PAAMATTLRKLLTGELLTASRQQLIDWMEADKVAGPLLR S ALPAGWFIADKSGAGERG S 240
MS210270_TEM      PAAMATTLRKLLTGELLTASRQQLIDWMEADKVAGPLLR S ALPAGWFIADKSGAGERG S 240
MS210416_TEM      PAAMATTLRKLLTGELLTASRQQLIDWMEADKVAGPLLR S ALPAGWFIADKSGAGERG S 240
*****

blaTEM_126_aa      RGIIAALGPDGKPSRIWIYTTGSQATMDERNRQIAEIGASLIKHW      286
MS170170_TEM      RGIIAALGPDGKPSRIWIYTTGSQATMDERNRQIAEIGASLIKHW      286
MS180027_TEM      RGIIAALGPDGKPSRIWIYTTGSQATMDERNRQIAEIGASLIKHW      286
MS190261_TEM      RGIIAALGPDGKPSRIWIYTTGSQATMDERNRQIAEIGASLIKHW      286
MS210270_TEM      RGIIAALGPDGKPSRIWIYTTGSQATMDERNRQIAEIGASLIKHW      286
MS210416_TEM      RGIIAALGPDGKPSRIWIYTTGSQATMDERNRQIAEIGASLIKHW      286
*****
```



blaTEM-143

CLUSTAL O(1.2.4) multiple sequence alignment

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blaTEM_143_RC      TTACCAATGCTTAATCAGTGAGGCACCTATCTCAGCGATCTGTCTATTTTCGTTCCATCCAT 60
MS170063_TEM      -----tcagtgaggcacctatctcagcgatctgtctatttcgttcatccat 46
                   *****

blaTEM_143_RC      AGTTGCCTGACTCCCCGTCGTGTAGATAACTACGATACGGGAGGGCTTACCATCTGGCCC 120
MS170063_TEM      agttgcctgactccccgtcgtgtagataactacgatacgggagggttaccatctggccc 106
                   *****

blaTEM_143_RC      CAGTGCTGCAATGATACCGCGAGACCCACGCTCACCGGCTCCAGATTTATCAGCAATAAA 180
MS170063_TEM      cagtgcctgcaatgataccgagaccacgctcaccggctccagatttatcagcaataaa 166
                   *****

blaTEM_143_RC      CCAGCCAGCCGGAAGGGCCGAGCGCAGAAGTGGTCCTGCAACTTTATCCGCCTCCATCCA 240
MS170063_TEM      ccagccagccggaaggccgagcgcagaagtggtcctgcaactttatccgcctccatcca 226
                   *****

blaTEM_143_RC      GTCTATTAATTGTTGCCGGAAGCTAGAGTAAGTAGTTCCGCCAGTTAATAGTTTGCGCAA 300
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                   *****

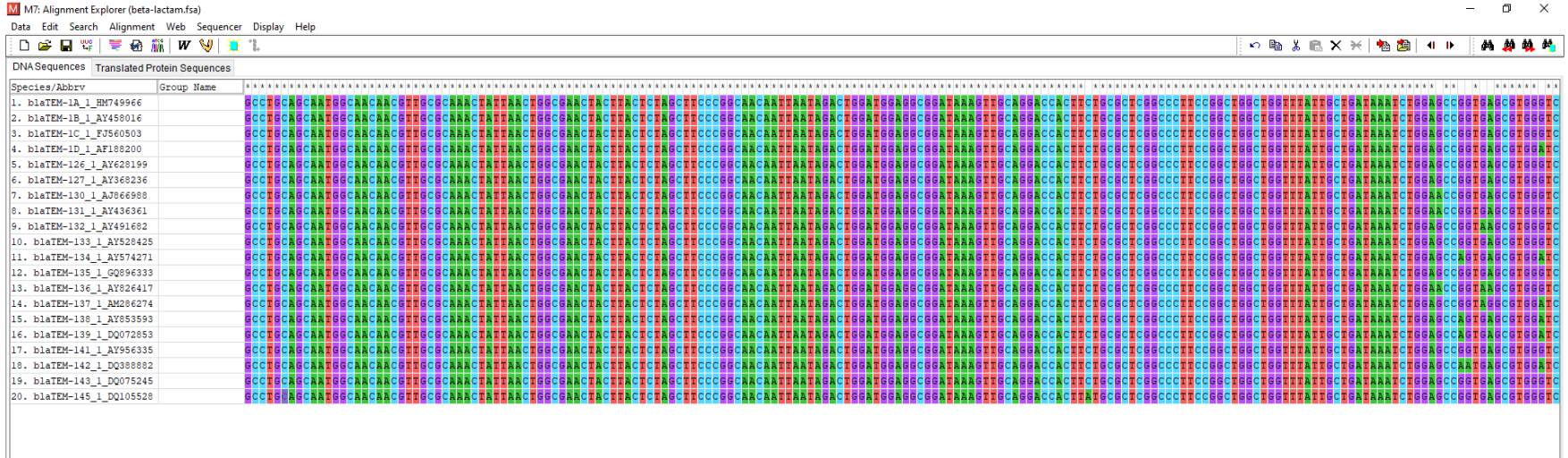
blaTEM_143_RC      CGTTGTTGCCATTGCTGCAGGCATCGTGGTGTACGCTCGTCGTTTGGTATGGCTTCATT 360
MS170063_TEM      cgttgttgccattgctgcaggcatcgtgggtgtcagctcgtcgnnttggatggcttcatt 346
                   *****

blaTEM_143_RC      CAGCTCCGGTCCCAACAATCAAGGCGAGTTACATGATCCCCCATGTTGTGCAAAAAGC 420
MS170063_TEM      cagctccggnnnnnnnnnnnnnnnngttacatgatcccccatgttgtgcaaaaagc 406
                   *****

blaTEM_143_RC      GGTTAGCTCCTTCGGTCCTCCGATCGTTGTGCAAGTAAGTTGGCAGCAGTGTATCACT 480
MS170063_TEM      ggttagctccttcggtcctccgatcgttgtcagaagtaagttggcagcagtggtatcact 466
                   *****
```



Mega



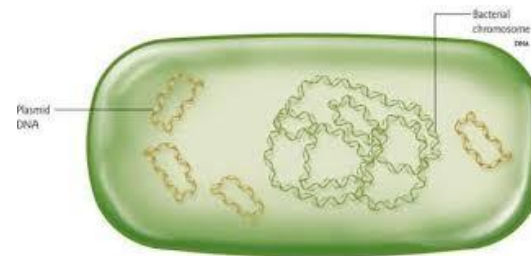
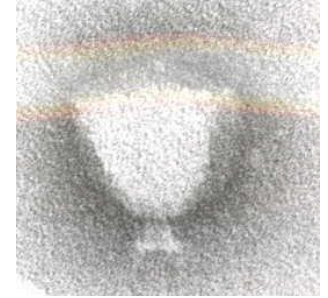
Many TEM enzymes vary by just one SNP

There exists large discrepancies in the functional subclass assignment (e.g. narrow-spectrum, ESBL etc.) of beta-lactamases across the various AMR databases.

Functional class assignment for the SHV beta-lactamases is particularly complicated because some intrinsic SHV alleles seem to have been mis-identified as causal variants of extended-spectrum or inhibitor resistances, due to the difficulties in distinguishing chromosomal and mobile variants.

Mobile Genetic Elements

- Plasmids
- Phages
- Transposons
- Integrons
- Insertion sequences

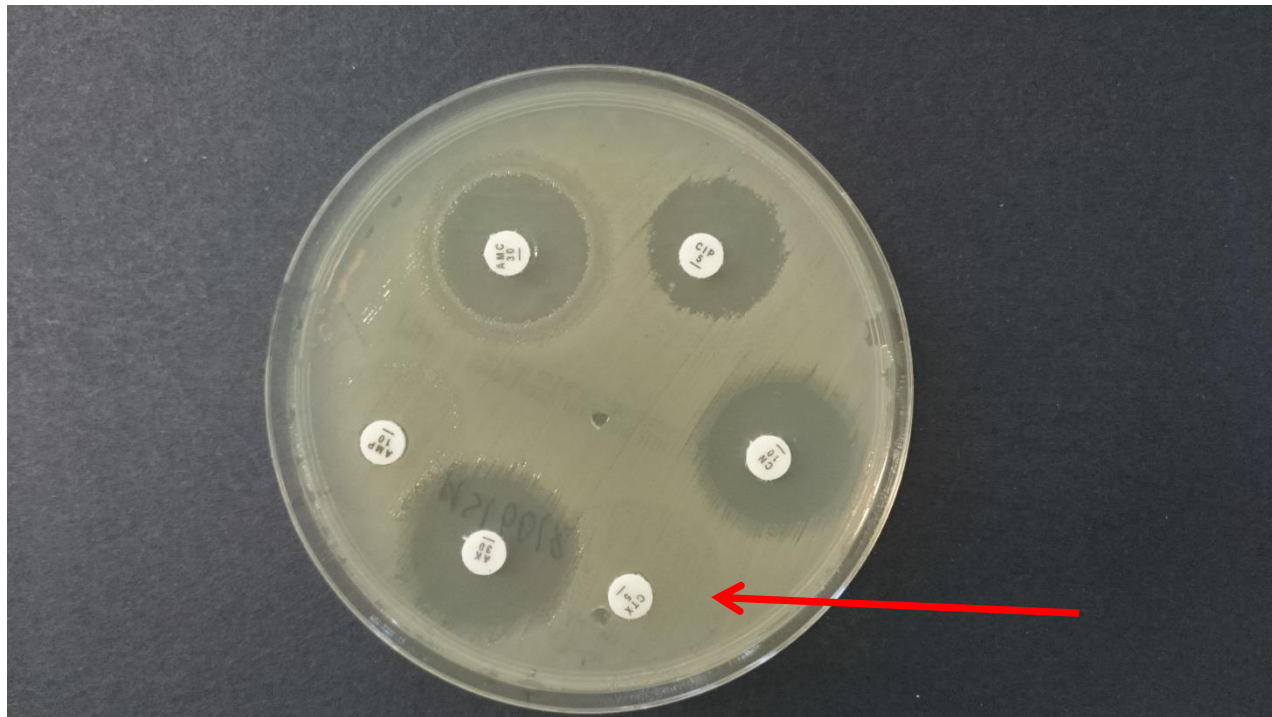


MDR ST13 S.Agona

ASuTmNaCpCazCtxAzt



Disk Diffusion



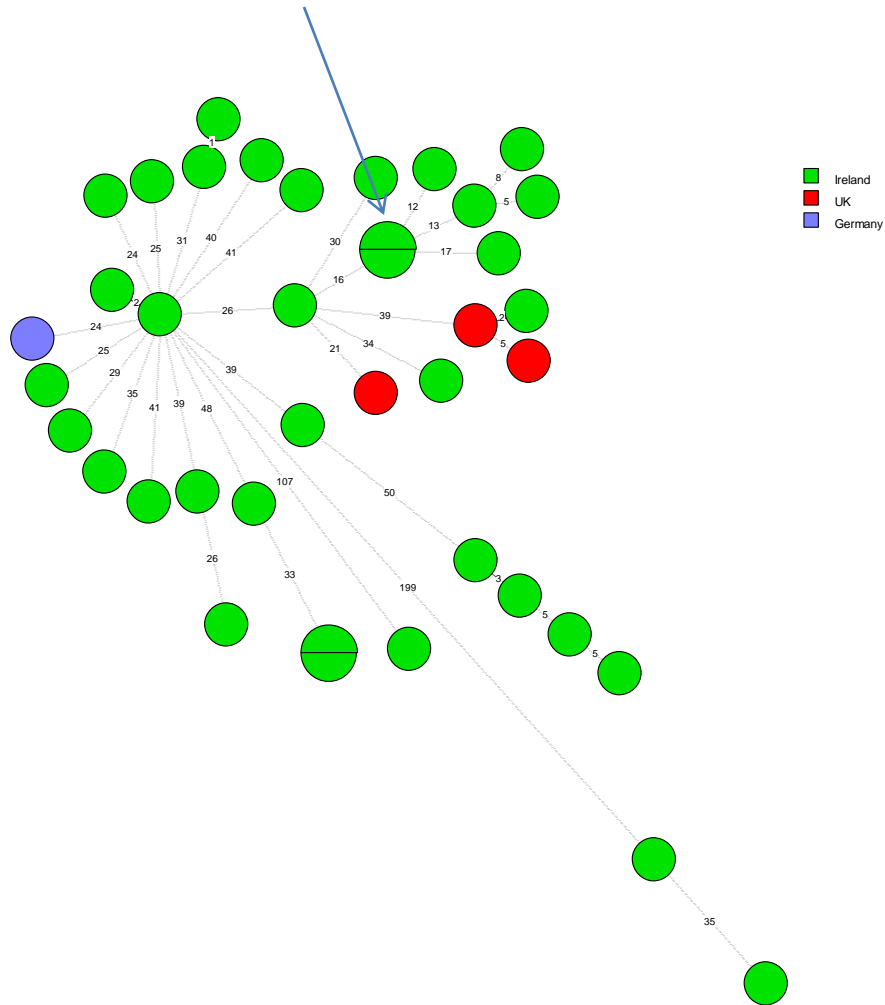
NaCp



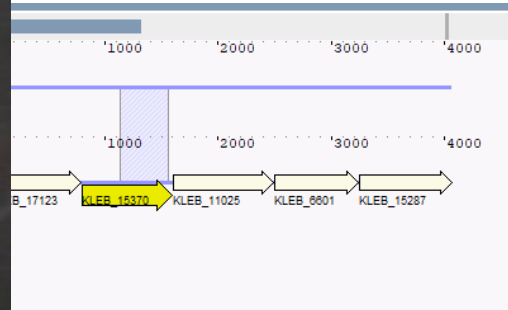
- Agona
 - ASuTmNaCpCazCtxAzt
 - *bla*CTX-M-15, *sul1*, *dfrA12*, *mph(A)*, *gyrA:p.D87Y*,
 - Plasmid IncL/M (96.69%), IncR (100%)

- Agona without Plasmid
 - NaCp
 - *gyrA:p.D87Y*

cgMLST of ST13 *S. Agona*



*bla*OXA-48 PCR pos ,WGS Neg



- *bla*OXA48 only present in <1% of cells

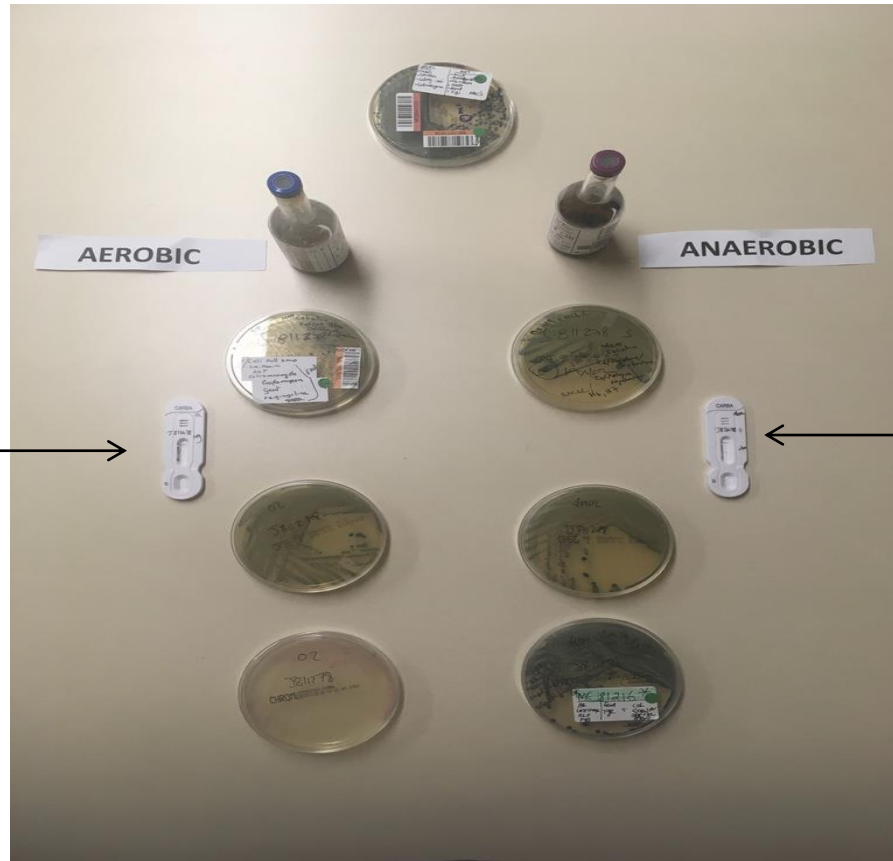
Now You See it, Now You Don't

Meropenem
MIC=0.25 mg/L

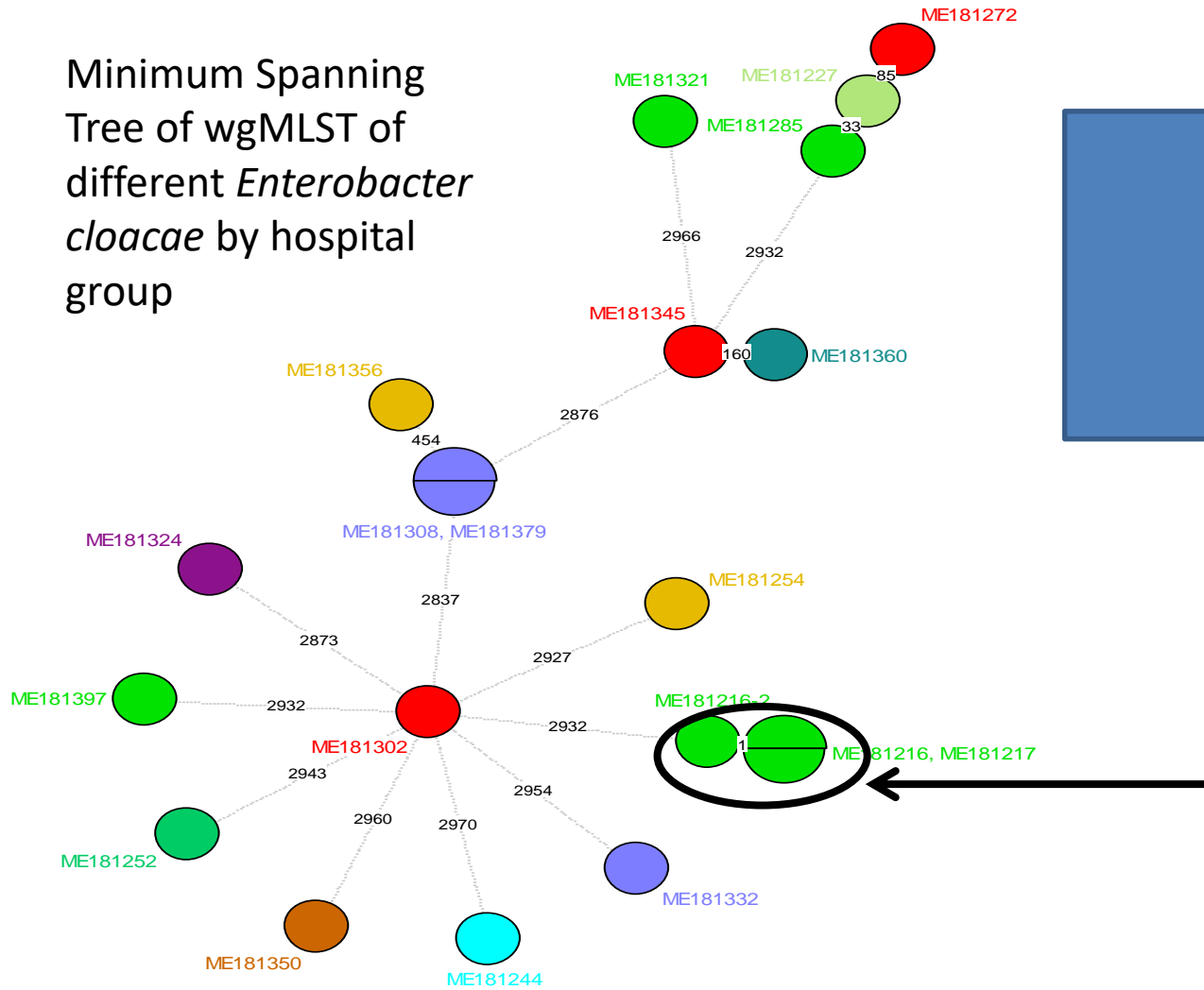
Meropenem
MIC=8mg/L

Negative

Positive
VIM



Minimum Spanning Tree of wgMLST of different *Enterobacter cloacae* by hospital group



Experiment data

Information fields

resistance

aac(6')-Ib
 blaVIM-1
 blaACT-7
 sul1
 aadA2
 aadB
 blaCTX-M-9
 dfrA16
 CnrA1
 oqxA
 oqxB
 tet(34)

Group	Key	Level
<input checked="" type="checkbox"/>	ME181216	
<input checked="" type="checkbox"/>	ME181216-2	

- Two resistance genes missing from isolate from aerobic bottle
 - blaVIM-1
 - aac(6')1b

Annual Report

Antimicrobial resistance

Following extensive validation, antimicrobial susceptibility patterns are now predicted from the whole genome sequence thus reducing analytical costs and laboratory waste.

More than half of the isolates (205 of 353, 58.1%) were susceptible to all antimicrobial agents tested. Multi-drug resistance (three or more different classes of antibiotics) was identified in 19.3% of isolates (n = 68), 27.9% of isolates that were multi-drug resistant, (n = 19) had the profile of resistance to ampicillin, sulphamide and tetracycline (ASuT) and were mainly monophasic *S. Typhimurium*.

A total of 6 extended spectrum beta-lactamase (ESBL) producing isolates were detected, all from patients with a history of recent foreign travel to Asia. These included 4 *bla*CTX-M-15 containing *S.Typhi*, all in blood cultures from patients with travel history to Pakistan; a *bla*CMY-2 isolate of *S.Typhimurium* (India) and a *S.Kentucky* with *bla*CMY-2 (Indonesia).

A total of 75 isolates had mutations in their *gyrA* and/or *parC* genes and/or encoded *qnr* genes which would result in reduced susceptibility to ciprofloxacin.

High level resistance to ciprofloxacin (>2mg/l) is rare among *Salmonella* but a ciprofloxacin-

GISAID for FWD Pathogens?

VOC Omicron GR/484A (B.1.1.529) first detected in Botswana/Hong Kong/South Africa

Map of tracked variant occurrence

Circle size proportional to number of variant genomes, zoom into region for more detail. Color by recency with red being most recent.

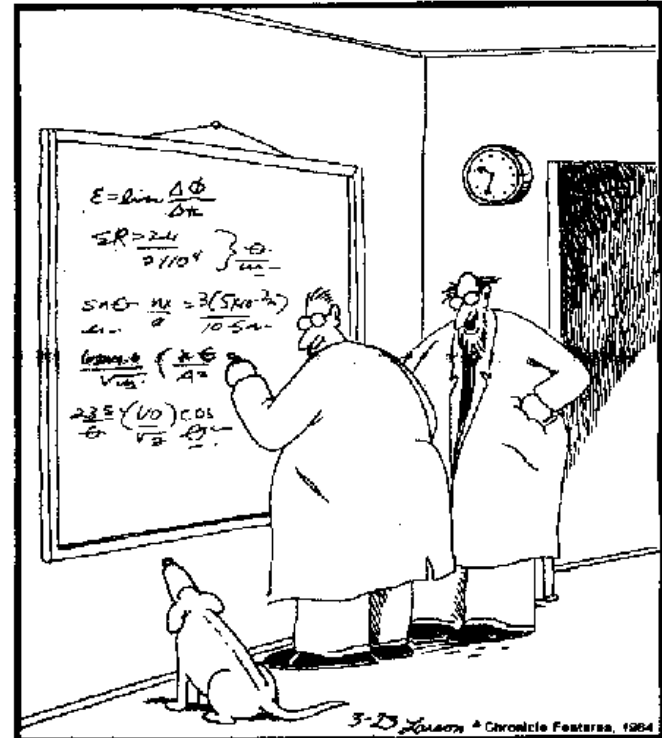


Acknowledgements

- Genevieve, Alma, Ger, Stuart, Martin

THE FAR SIDE

By GARY LARSON



"Ohhhhhh . . . Look at that, Schuster . . .
Dogs are so cute when they try to comprehend
quantum mechanics."