Application of learnings from *bla*$_{\text{CTX-M}}$ to characterization of plasmids harboring antimicrobial-resistance genes.

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Introduction

- ✓ Worldwide-distributing antimicrobial-resistant bacteria are serious public health concerns.
- ✓ Extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae is one of the CDC's serious threats.
- ✓ We have observed more than 50% of asymptomatic healthy individuals in the Southeast Asian countries carried ESBL-producing bacteria.

Introduction

- ✓ Acquiring antimicrobial-resistance (AMR) gene(s) and/or gene mutations are main cause of emerging of these bacteria. Such as, *Escherichia coli* that acquired *bla*$_{CTX-M}$ was transformed to ESBL-producing *E. coli*.
- ✓ CTX-M type ESBL gene, *bla*$_{CTX-M}$ is transported among bacterial cells via plasmid harboring multiple AMR genes.
- ✓ In adiition, *bla*$_{CTX-M}$ is one of the IS-transported AMR genes; therefore, *bla*$_{CTX-M}$ is found both on plasmid(s) and chromosome.

1st Aim

- ✓ To observe what types of ESBL-producing bacteria asymptomatic healthy people carried in Vietnam and Indonesia.

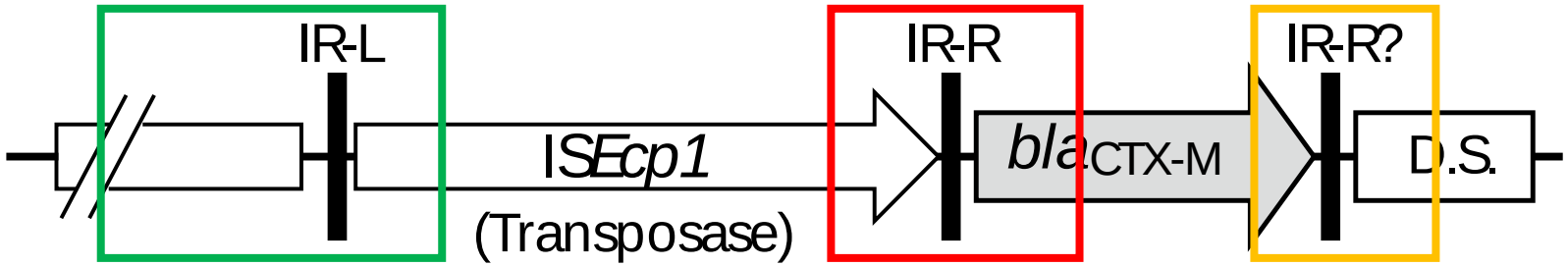
Ideas or strategies

- ✓ To establish analytical method to discriminate location of *bla*$_{CTX-M}$.
- ✓ To establish genotyping method based on the *bla*$_{CTX-M}$ location.

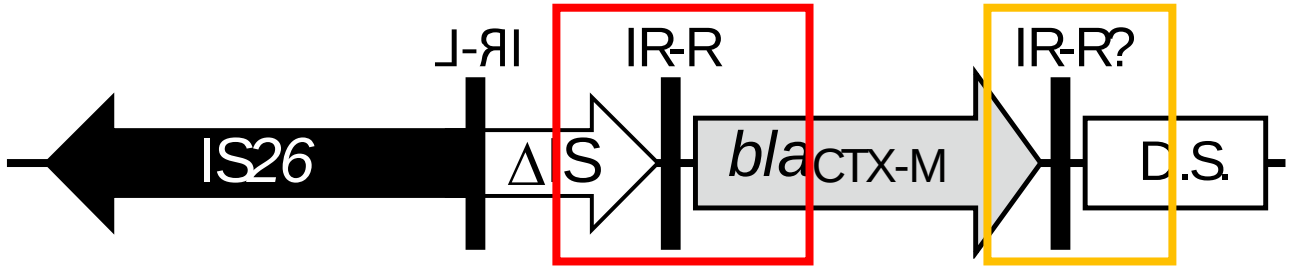
Molecular structure of *bla*$_{CTX-M}$ transposition unit.

Next generation sequencer is not suitable for determining *bla*$_{CTX-M}$ transposition unit.

Original structure

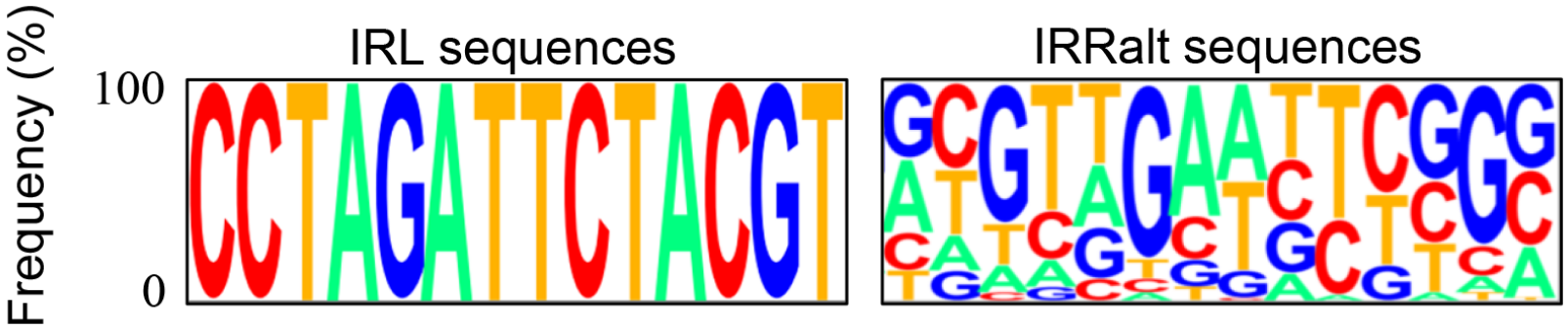


Disrupted structure (often found on plasmid)



1st Learning

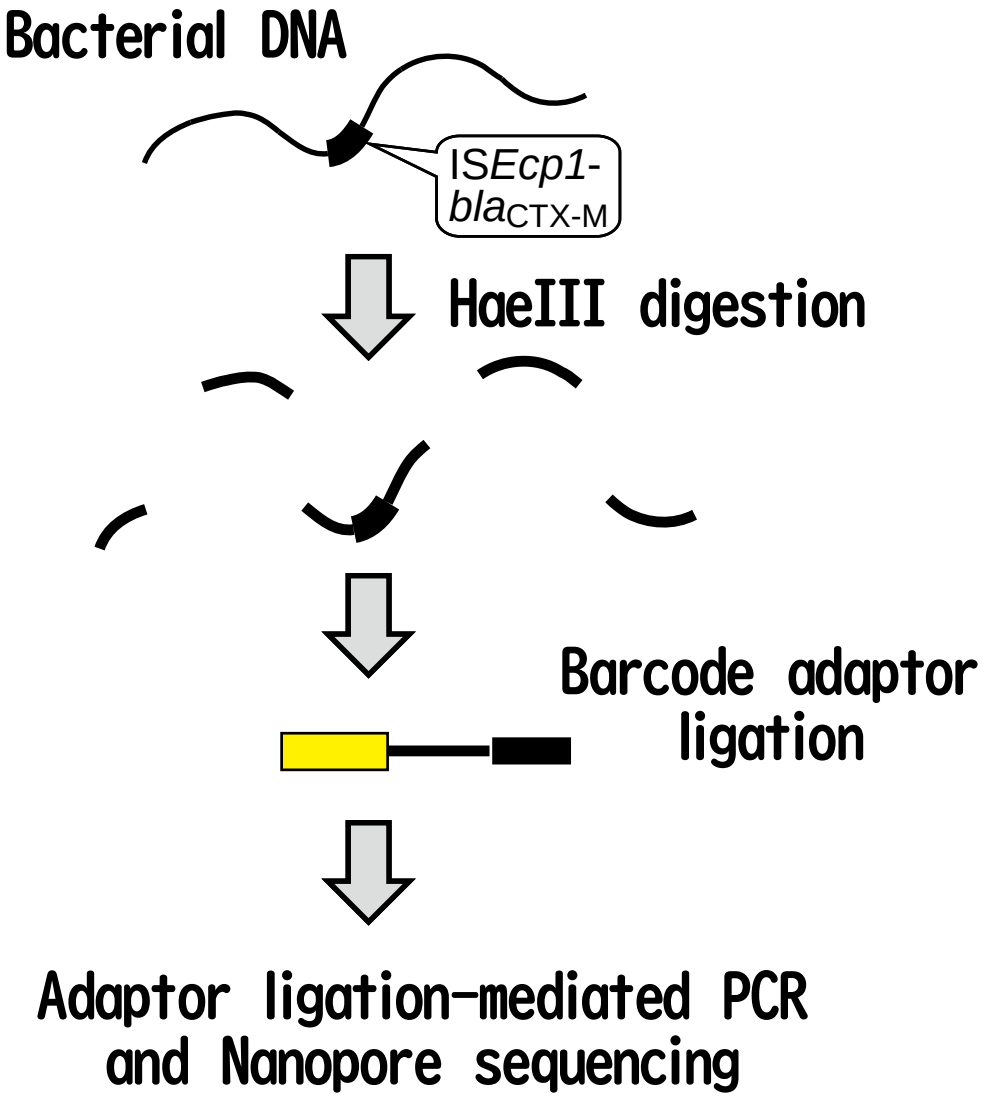
Observations of bacterial isolates and model *E. coli* strain indicated that IR-L (upstream) of *bla*$_{CTX-M}$ transposition unit is well conserved.



This results implied us a possibility of genotyping method based on the upstream genetic structure of *bla*$_{CTX-M}$.

Hamamoto K, *et al.*, Int J Med Microbiol. 2020, 310(2):151395.

Based on the 1st learning, we established a genotyping method.



- ✓ As shown in the left figure, genomic DNA is digested by restriction enzyme, HaeIII and subjected to adaptor ligation mediated PCR.
- ✓ Amplified DNA was subjected to the Nanopore sequencer.
- ✓ Upstream genetic structure (UGS) of *bla*$_{CTX-M}$ was used for genotyping and classification.

Yagi N, *et al.,* J Infect Chemother., 2021, 27(9), 1288-1294.
Widyatama FS, *et al*., Microbiol Immunol., 2021, 65(12), 542-550.

Then we analyzed 501 isolates from Vietnam and Indonesia.

UGS type #	Isoates sources	Detected number			Ratio (%)			*bla* CTX-M group	Location[1]	IS*Ecp1* [2]	Disrupted site[3]	BioSample #
		Total	IDN	VNM	Total	IDN	VNM					
101	IDN	56	56	0	11.2	30.9	0.0	1	Plasmid	disrupted	24	SAMD00272437
102	IDN&VNM	38	27	11	7.6	14.9	3.4	1	Plasmid	disrupted	1370	SAMD00272503
103	VNM	21	0	21	4.2	0.0	6.6	1	Plasmid	intact		SAMD00272645
104	VNM	21	0	21	4.2	0.0	6.6	1	Plasmid	disrupted	1382	SAMD00272331
105	VNM	19	0	19	3.8	0.0	5.9	1	Plasmid	disrupted	1227	SAMD00272170
106	IDN	12	12	0	2.4	6.6	0.0	1	Plasmid	intact		SAMD00272571
107	IDN	7	7	0	1.4	3.9	0.0	1	Chromosome	intact		SAMD00272424
108	VNM	7	0	7	1.4	0.0	2.2	1	Chromosome	intact		SAMD00272539
109	VNM	6	0	6	1.2	0.0	1.9	1	Plasmid	intact		SAMD00272308
110	VNM	6	0	6	1.2	0.0	1.9	1	Plasmid	disrupted	-38	SAMD00272268
111	VNM	6	0	6	1.2	0.0	1.9	1	Plasmid	disrupted	253	SAMD00272341
112	IDN&VNM	5	4	1	1.0	2.2	0.3	1	Chromosome	intact		SAMD00272244
113	IDN	5	5	0	1.0	2.8	0.0	1	Chromosome	intact		SAMD00272479
114	VNM	5	0	5	1.0	0.0	1.6	1	Plasmid	intact		SAMD00272249
115	IDN	5	5	0	1.0	2.8	0.0	1	Plasmid	disrupted	973	SAMD00272412
116	IDN&VNM	4	3	1	0.8	1.7	0.3	1	Chromosome	intact		SAMD00272195
117	IDN	4	4	0	0.8	2.2	0.0	1	Chromosome	intact		SAMD00272431

2nd learning

The results obtained from the analysis.

- ✓ In total 502 UGS of *bla*$_{CTX-M}$ were obtained from 501 isolates.
- ✓ The 502 UGSs were classified into 85 UGS types.
- ✓ Among the 85 UGS types, 6 UGS types were commonly observed in the two countries.
- ✓ In 71.5% of the UGSs, IS*Ecp1* was disrupted, suggesting *bla*$_{CTX-M}$ would not be transferrable.
- ✓ Most (87.1%) of the UGSs were plasmidic.

These results encouraged us to application of UGS analysis to other AMR genes.

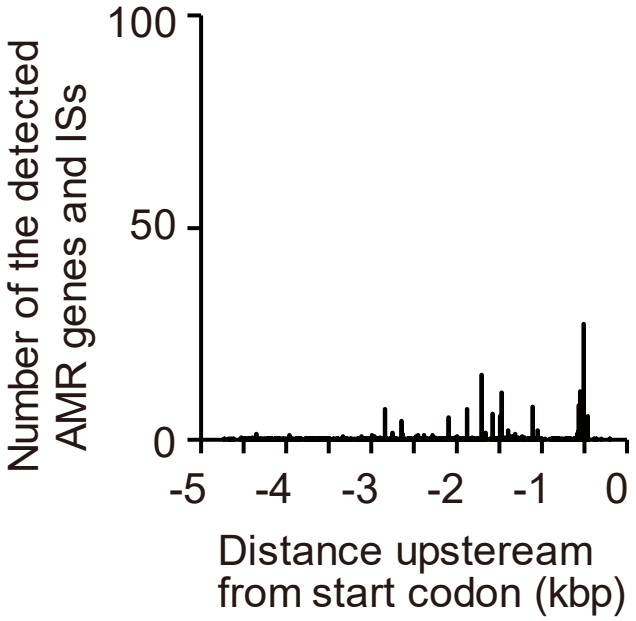
2nd Aim (application)

- ✓ To establish genotyping method based on the location of AMR genes' location.
- ✓ To consider UGSs of AMR genes to presuming plasmid(s) harboring AMR genes.

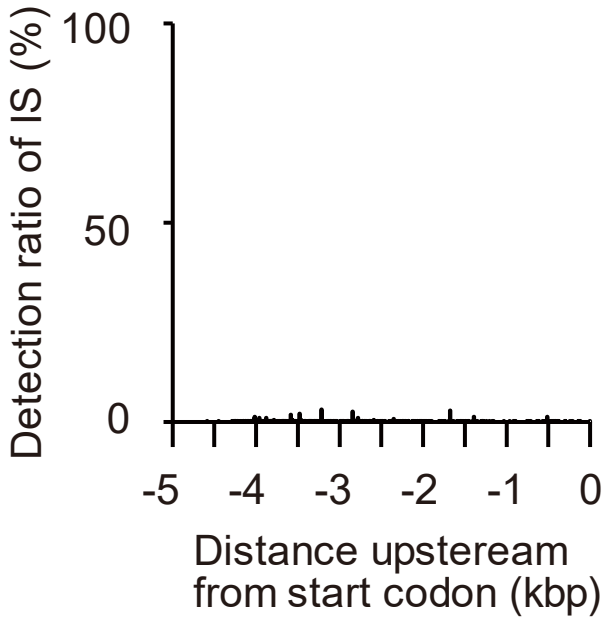
How we applied the learnings to other AMR genes.

- ✓ We retrieved 51,344 bacterial plasmid sequences from the database, RefSeq, and selected 9,048 plasmids containing at least one AMR gene of the top 30 detected AMR genes.
- ✓ We analyzed upstream region of the top 30 detected AMR genes up to 5,000 base pare length.
- ✓ We performed *in silico* UGS analysis for the top 30 detected AMR genes.
- ✓ We are considering how we can apply the UGSs of AMR genes to presume AMR plasmid(s).

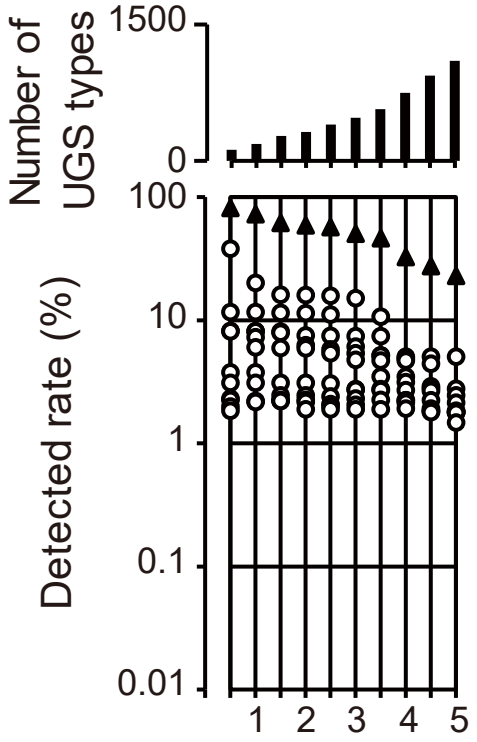
One example of our analysis.



Other AMR gene



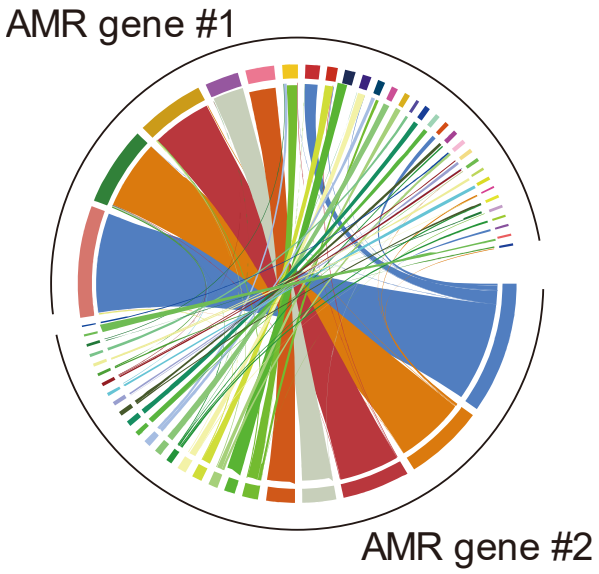
IS



in silico UGS typing

What we are learning.

- ✓ UGSs of the top 30 detected AMR genes could be typable as same as *bla*$_{CTX-M}$, even though there are many UGS types.
- ✓ There were certain combinations of UGS types of certain two of the top 30 AMR genes.
- ✓ There is a possibility that UGS types can be a good keys to search reference plasmid sequence.



- ✓ There is a possibility that UGS types can be a good keys to search reference plasmid sequence.

Plasmid (NZ_CP041032.1)

AMR gene	UGS	ORF
bla$_{CTX-M-15}$	43	>1000
sul1	511	>1000
tet(A)	871	>1000
mph(A)	316	>1000
aac(6')-Ib-cr5	459	>1000
catB3	678	>1000
bla$_{OXA-1}$	529	>1000
dfrA17	44	>1000
aadA5	186	>1000

AMR gene	UGS	ORF
bla$_{CTX-M-15}$ + *sul1*	27	230
bla$_{CTX-M-15}$ + *tet(A)*	12	296
bla$_{CTX-M-15}$ + *mph(A)*	28	221
bla$_{CTX-M-15}$ + *aac(6')-Ib-cr5*	12	499
bla$_{CTX-M-15}$ + *catB3*	31	436
bla$_{CTX-M-15}$+ *bla*$_{OXA-1}$	13	437
bla$_{CTX-M-15}$+ *dfrA17*	2	76
bla$_{CTX-M-15}$+ *aadA5*	2	75
bla$_{CTX-M-15}$ + *tet(A)* + *dfrA17*	2	29
bla$_{CTX-M-15}$ + *mph(A)* + *dfrA17*	2	74
bla$_{CTX-M-15}$+ *bla*$_{OXA-1}$ + *dfrA17*	2	83

Summary

- ✓ At least, UGSs of AMR genes could be used for genotyping and hopefully homology search as “keys”.
- ✓ Bacterial whole genome sequencing will be more actively performed. Most difficult part is determination of plasmid structure.
- ✓ If we could use UGSs as the keys, plasmid analysis could be done efficiently, because you could find the reference sequence easier than it is now.