

Potential Transmission of antimicrobial-resistant bacteria derived from livestock via vegetables in Japan

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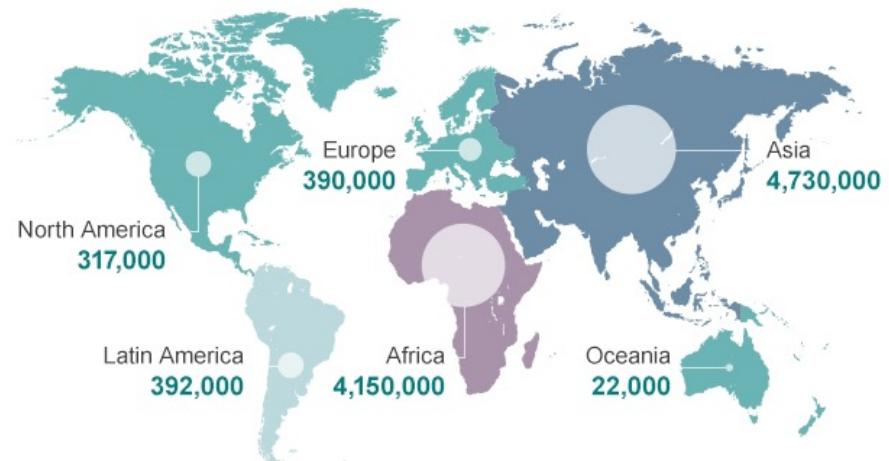
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Antibiotic resistance is worldwide concern

- **Antibiotic resistance** was **top of the agenda** at a meeting of international science ministers in the **G8 summit** (UK, 2013).
- It has been predicted to lead to **10 million casualties annually by 2050**.

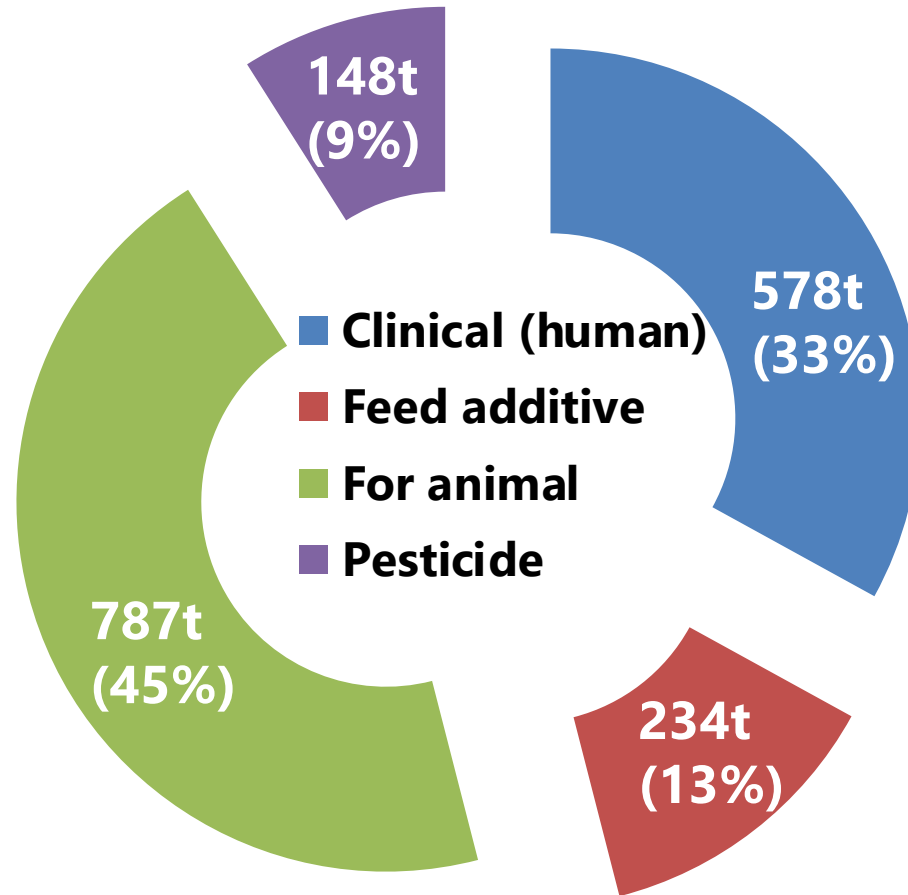
Deaths attributable to antimicrobial resistance every year by 2050



Source: Review on Antimicrobial Resistance 2014

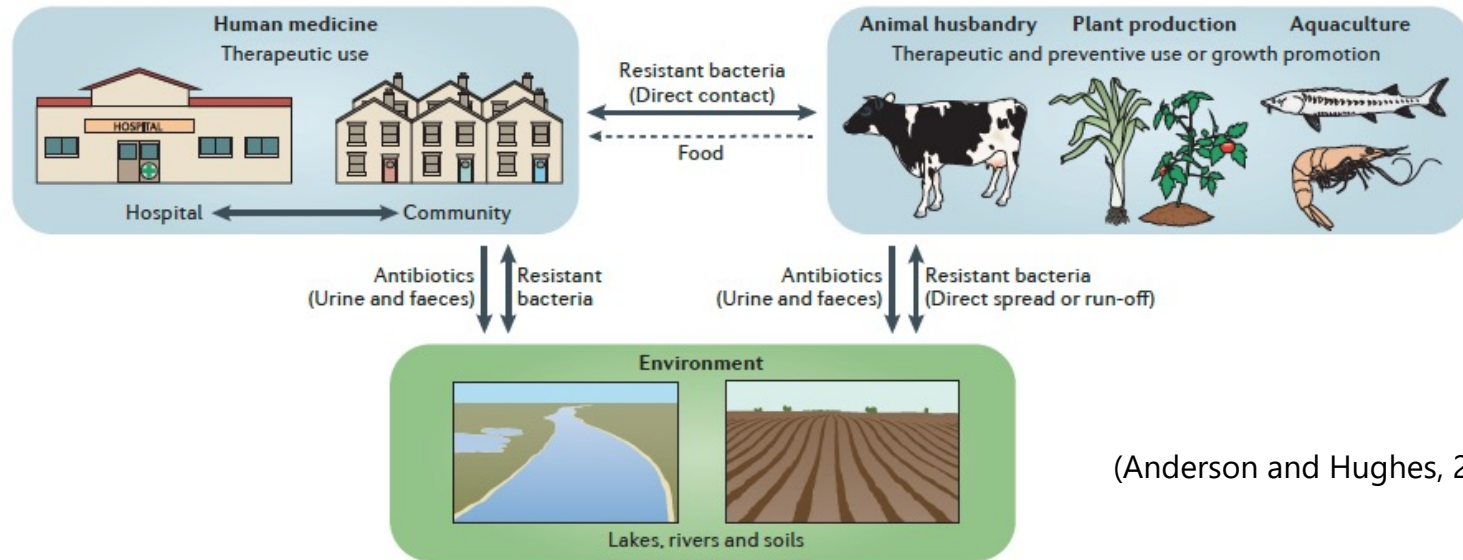
Some counter measures against antibiotic resistance need to be taken quickly.

The amount usage of antibiotics in Japan



Many antibiotics are used for animals.

Antibiotic resistant bacteria (**ARB**) and antibiotic resistance genes (**ARGs**) are spreading from animals to humans.



(Anderson and Hughes, 2014)

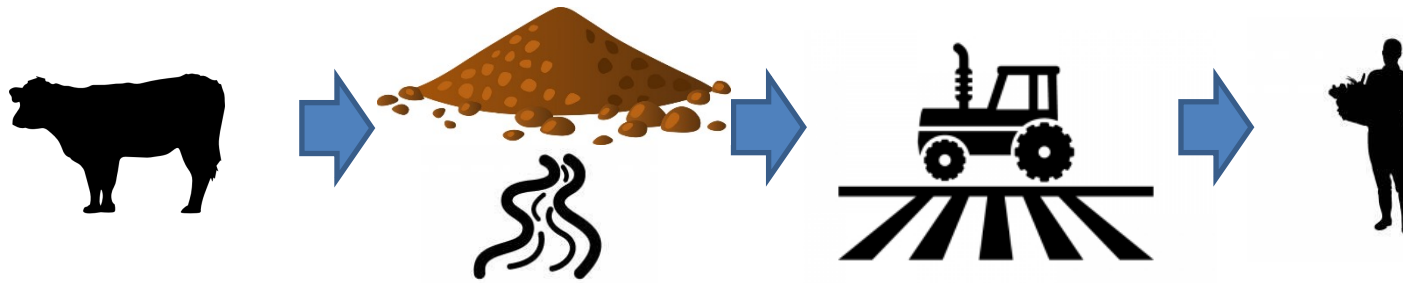
- **ARB were detected in commercial Vegetables**
- **Since farm manure and wastewater are used in the field, ARB derived from livestock may be one of the sources of ARB isolated from vegetables**
- **Vegetables are often consumed raw, and there is a possibility of spreading ARB to humans**

Today's topics

(1) The transmission degree of ARB in vegetables derived from livestock in university-affiliated farm

(2) Genome analysis of ARB derived from cattle, aquatic environments, vegetables, and human clinical sources

Elucidation of the amount of ARB transmission from farms to vegetables



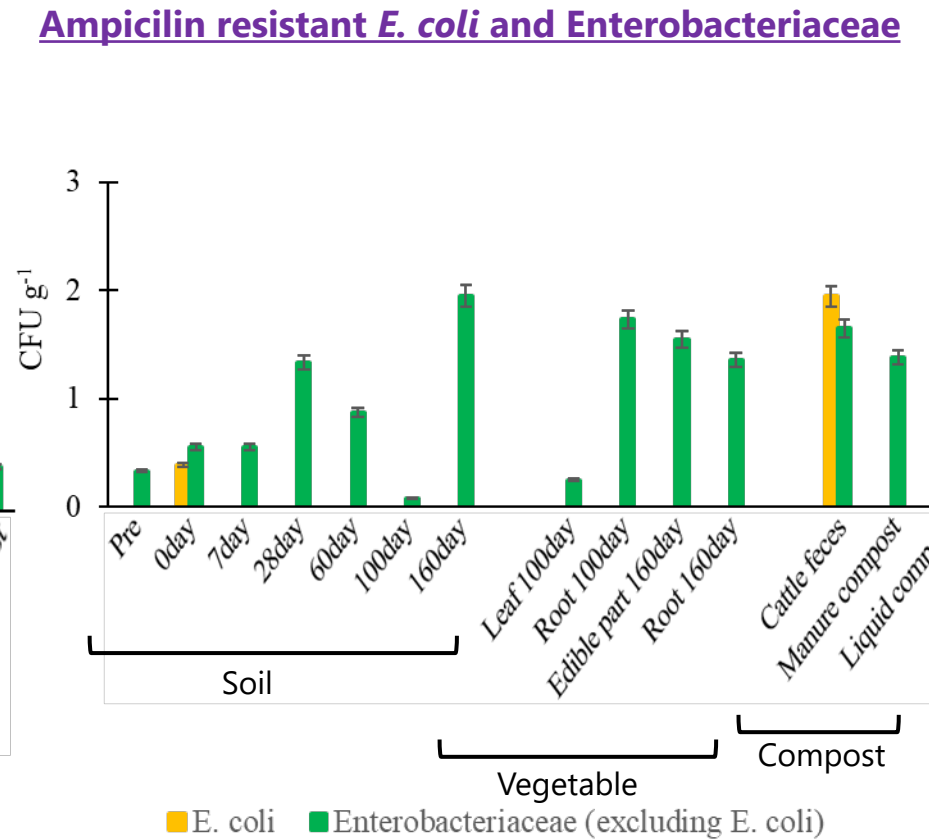
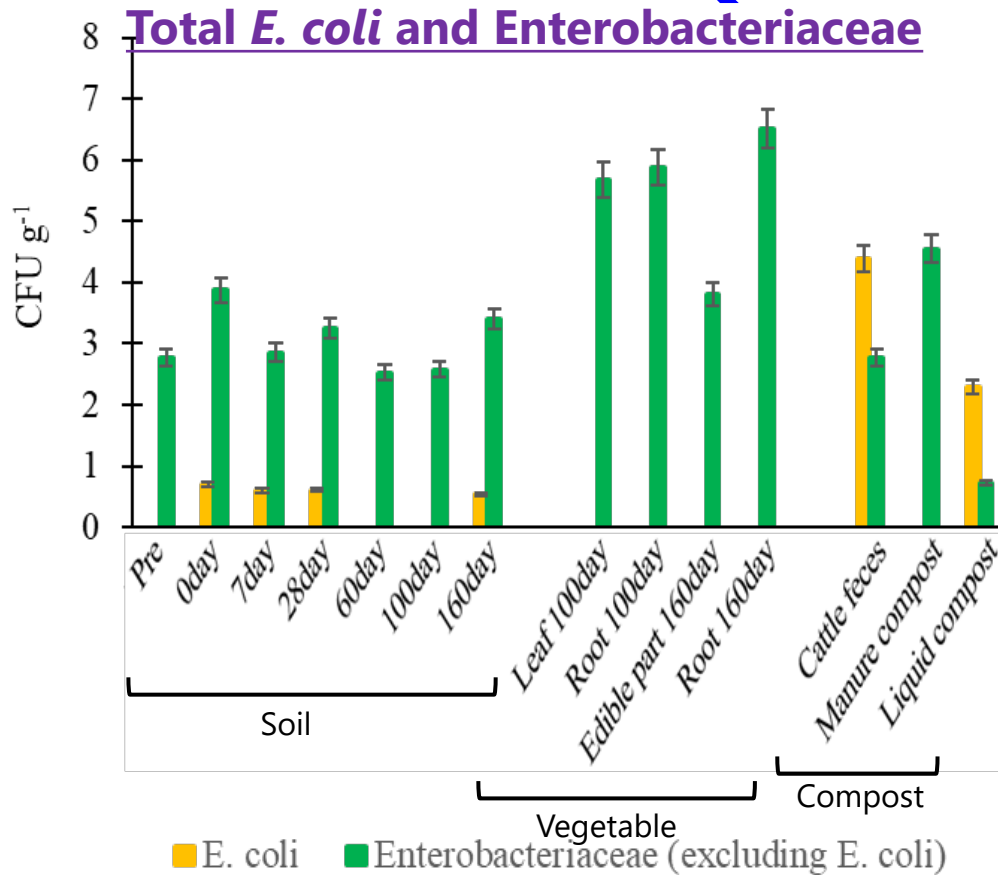
Follow livestock to vegetables in the same field

Sample: Cattle feces, compost, wastewater, livestock waste spread soil, and vegetables

Quantification and isolation of *E. coli* and ampicillin-resistant bacteria

Clarifying the extent of transmission of ARB from livestock to soil and vegetables.

Quantification

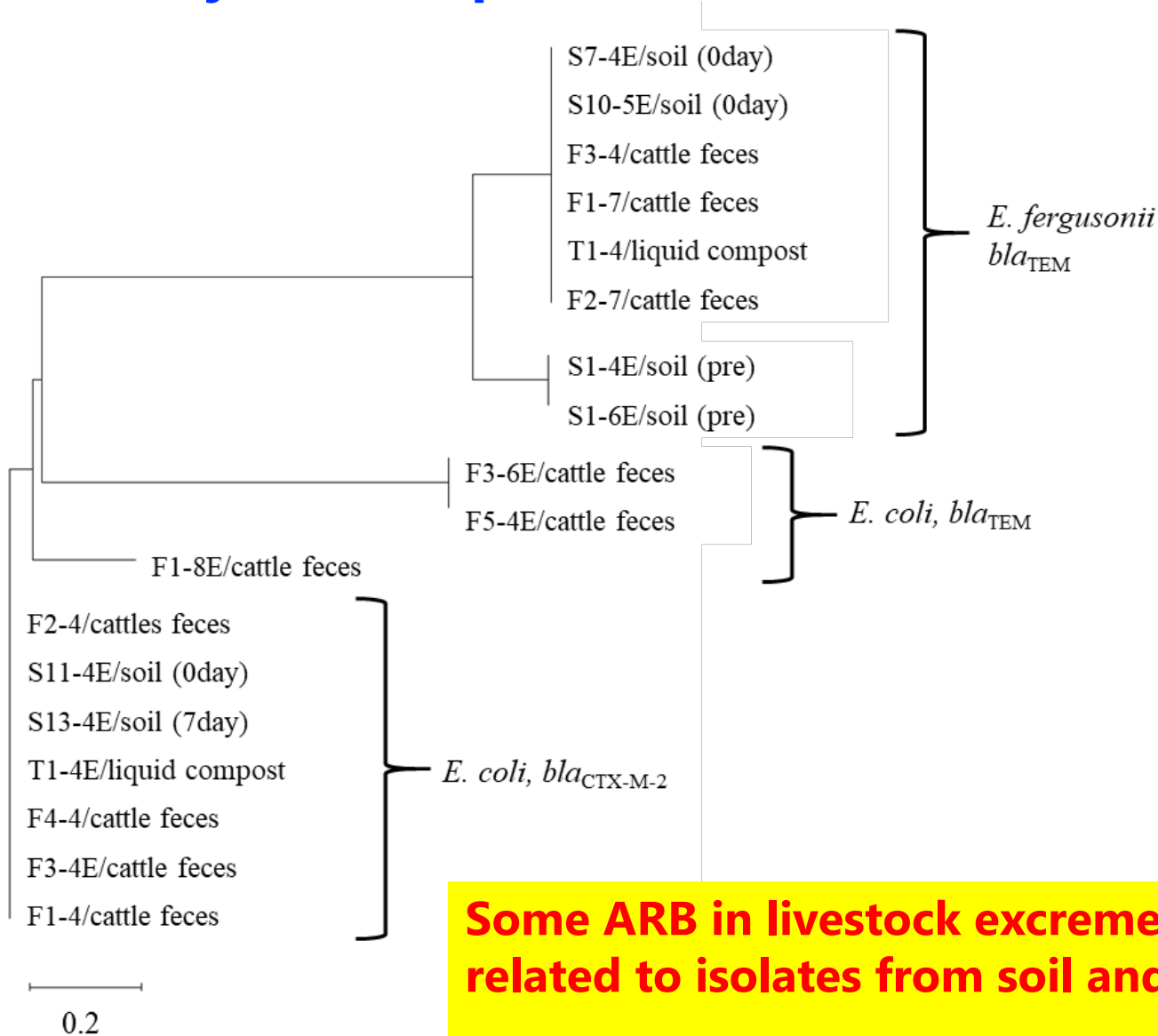


The abundance of *E. coli* and Enterobacteriaceae were increased in soil immediately after application of compost.

The abundance of *E. coli* in manure compost is very low, because these compost is well composted

After application of compost, the abundance of bacteria were gradually decreases

NGS analysis of ampicillin resistant bacteria



Some ARB in livestock excrement are closely related to isolates from soil and crops

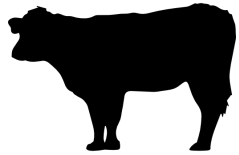
ARB derived from livestock could transmit to soil and crops

Today's topics

(1) The transmission degree of ARB in vegetables derived from livestock in university-affiliated farm

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Genome analysis of ARB derived from cattle, aquatic environments, vegetables, and human clinical sources



Cattle, pig



Waste water, river

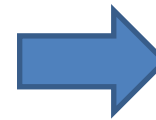
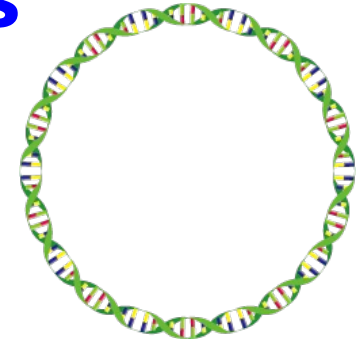


Vegetables



Human clinical

Isolation of ampicillin resistant *E. coli* and bacteria



Focus on beta lactamase







Clarifying the relationship of ampicillin resistant bacteria

Isolation results from vegetables

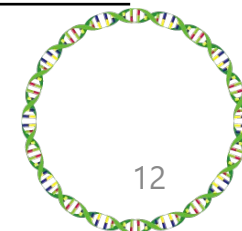
- 200 vegetables
- 1142 strains were isolated
- *E. coli* were isolated from 5 samples (positive rate of *E. coli* is 2.5%) (all *E. coli* strains are not antibiotic resistance)
- **Contamination rate of *E. coli* in commercial vegetables in Japan is low.**

739 strains of ampicillin resistant bacteria were collected

bla typing

Origin	number	TEM	SHV	CTX-M1	CTX-M9
 Cattle feces	18	11	ND	ND	7
Compost	15	10	ND	5	ND
Cattle feces	86	72	1	1	1
 Sick cattle	45	40	1	2	5
Pig feces	116	112	0	0	3
Livestock waste	25	25	ND	ND	ND
 Waste water	6	6	ND	ND	ND
Slaughter house	6	6	ND	ND	ND
Dairy waste water	14	11	ND	3	ND
WWTP	55	22	4	19	19
 Vegetable	148	19	5	76	76
Human clinical	205	121	1	10	48

Whole genome analysis for TEM-positive strains (focus on plasmids harboring TEM)



Draft genome sequence analyzed for contigs containing blaTEM-1 (332 contigs)



211/332 contigs have mobile genetic elements
(MobileElementFinder : identity $\geq 90\%$, coverage $\geq 60\%$)

e.g. **IS26 : 47**

IS30 : 5

IS5075: 23

Tn2: 118

Tn10: 5

Tn1000: 6

Tn1721: 6

Tn4656: 7

Tn1000: 6

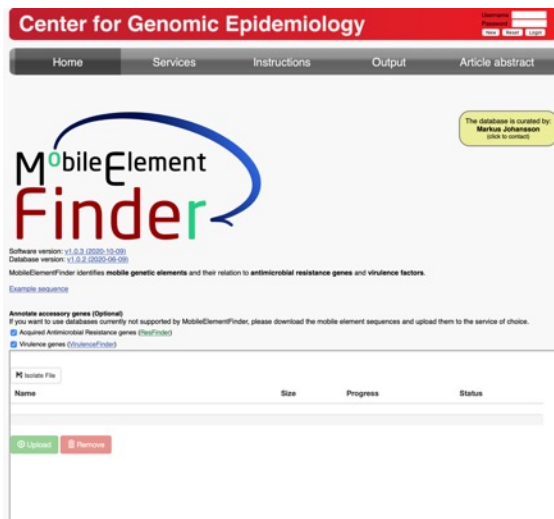
ISEc37: 5

ISKpn19 : 4

ISVsa3: 5

ISVsa5: 7

-> Detailed analysis for IS26-positive strains



IS26 & *bla*_{TEM-1} positive bacteria (47 strains)

Source



Source	Isolate	Species	HLST	Group	contig	ABC	MGE	Plasmid region	Length
NVAL	DB36	ecoli	744	A	DB36_contig00063	aadA5, aph(3*)-Ib, aph(6)-Id, blaTEM-1B, catA1, dfrA17, mph(A), qacE, sul1, sul2	IS26, IS26, IS26, IS6100, Tn6205	IncQ1	24,950
NVAL	P114	ecoli	10	A	P114_contig00194	aph(3*)-Ib, aph(6)-Id, blaTEM-1B, dfrA8, sul2	IS26, Tn6205	None	8,484
NVAL	P4	ecoli	10	A	P4_contig00143	aph(3*)-Ib, aph(6)-Id, blaTEM-1B, dfrA14, sul2	IS26, IS5075, Tn6205	None	11,144
NVAL	PS4	ecoli	1244	A	PS4_contig00105	blaTEM-1B, flrR	IS26, IS903	None	9,155
GU	PFABPC11	ecoli	542	A	PFABPC11_contig00048	blaTEM-1B, sul3	IS26, IS26, Tn2	None	17,310
RGU	163	ecoli	937	B1	163_contig00037	blaTEM-1D	IS26	None	41,843
NVAL	836	ecoli	642	B1	836_contig00182	blaTEM-1B	IS26	None	1,935
NVAL	849	ecoli	164	B1	849_contig00079	aph(3*)-Ib, aph(6)-Id, blaTEM-1A, flrR, sul2, tet(A)	IS26, ISVna3, Tn1000, Tn6205	None	17,857
NVAL	863	ecoli	164	B1	863_contig00072	aph(3*)-Ib, aph(6)-Id, blaTEM-1A, sul2, tet(A)	IS26, Tn1000, Tn6205	None	15,818
NVAL	866	ecoli	164	B1	866_contig00080	aph(3*)-Ib, aph(6)-Id, blaTEM-1A, flrR, sul2, tet(A)	IS26, ISVna3, Tn1000, Tn6205	None	17,938
NVAL	B7	ecoli	58	B1	B7_contig00188	blaTEM-1B	IS26, Tn2	None	6,396
NVAL	881	ecoli	1704	B1	881_contig00112	aph(3*)-Ib, aph(6)-Id, blaTEM-1A, sul2, tet(A)	IS26, Tn1000, Tn6205	None	13,541
HU	CE-1-2	ecoli	4623	B1	CE-1-2_contig00047	blaAP-2, blaTEM-1B, qnrS1, tet(A)	IS26, IS30, ISKpn19	None	30,345
NVAL	DB26	ecoli	56	B1	DB26_contig00101	blaTEM-1B, dfrA17	IS26, Tn2	None	13,946
NVAL	DB37	ecoli	101	B1	DB37_contig00033	aph(3*)-Ib, aph(6)-Id, blaTEM-1B, sul2, tet(A)	IS26, IS26, IS5075, Tn6205	IncFIA, IncFIC(FII)	43,168
NVAL	DB38	ecoli	224	B1	DB38_contig00114	aac(3)-IId, blaTEM-1B	IS26	None	3,297
NVAL	DB42	ecoli	58	B1	DB42_contig00064	blaTEM-1B	IS26, Tn4656	None	22,477
NVAL	DB43	ecoli	2329	B1	DB43_contig00070	blaTEM-1B	IS26, Tn2	IncFIB(AP001918)	16,105
GU	GABPCS	ecoli	795	B1	GABPCS_contig00025	blaTEM-1B, sul2	IS26, IS679, ISVna5	IncFII	58,643
HU	MA-1-1b	ecoli	7937	B1	MA-1-1b_contig00054	aac(3)-IId, aadA2, blaTEM-1B, catA1, dfrA12, mph(A), qacE, sul1	IS26, IS6100, ISCFr1	IncFII(ANMA1167-NDM-5)	32,844
NVAL	P104	ecoli	101	B1	P104_contig00053	aph(3*)-Ib, aph(6)-Id, blaTEM-1B, sul2, tet(A)	IS26, IS5075, Tn2, Tn6205	IncFIC(FII)	28,353
NVAL	P20	ecoli	58	B1	P20_contig00030	aph(3*)-Ib, aph(6)-Id, blaTEM-1B, sul2	IS26, IS5075, Tn2, Tn6205	IncFIC(FII)	32,967
NVAL	P64	ecoli	1727	B1	P64_contig00034	aph(3*)-Ib, aph(6)-Id, blaTEM-1B, dfrA14, sul2, tet(A)	IS26, IS26, IS5075, Tn2, Tn6205	IncFIC(FII)	35,289
NVAL	P80	ecoli	641	B1	P80_contig00077	aadA1, blaTEM-1B, dfrA1, sul3	IS26	None	20,425
SMU	ABPCE11_2	ecoli	131	B2	ABPCE11_2_contig00126	blaTEM-1B	IS26, Tn2	None	11,963
SMU	ABPCE112	ecoli	131	B2	ABPCE112_contig00056	blaTEM-1B	IS26	IncFIB(AP001918)	9,976
SMU	ABPCE129	ecoli	131	B2	ABPCE129_contig00160	blaTEM-1B	IS26, IS629	None	6,658
SMU	ABPCE141	ecoli	131	B2	ABPCE141_contig00040	blaTEM-1B	IS26	None	17,126
SMU	ABPCE170	ecoli	1193	B2	ABPCE170_contig00057	blaTEM-1B	IS26	None	6,921
SMU	ABPCE24	ecoli	569	B2	ABPCE24_contig00065	aph(3*)-Ib, aph(6)-Id, blaTEM-1B, sul2	IS26, IS5075, Tn2, Tn6205	IncFIC(FII)	28,484
SMU	ABPCE5	ecoli	131	B2	ABPCE5_contig00093	blaTEM-1B	IS26, IS629	None	16,396
SMU	ABPCE51	ecoli	131	B2	ABPCE51_contig00078	aac(3)-IId, blaTEM-1B	IS26, ISCFr1	IncFII(pCoo)	16,601
SMU	ABPCE81	ecoli	131	B2	ABPCE81_contig00131	blaTEM-1B	IS26	None	10,948
SMU	ABPCE9	ecoli	73	B2	ABPCE9_contig00063	aac(3)-IId, blaCTX-M-3, blaTEM-1B	IS26, ISCFr1	None	9,752
SMU	ABPCE91	ecoli	12	B2	ABPCE91_contig00047	ant(3*)-Ib, blaTEM-1B, qacE, sul1	IS26, ISKpn26, Tn2, Tn21	None	32,490
SMU	ABPCE97	ecoli	N/A	B2	ABPCE97_contig00168	blaTEM-1B	IS26	None	3,746
SMU	ABPCE98	ecoli	131	B2	ABPCE98_contig00232	blaTEM-1B	IS26	None	2,630
SMU	ABPCE1_1	ecoli	69	D	ABPCE1_1_contig00158	blaTEM-1B	IS26	None	8,319
RGU	F3-6E	ecoli	973	D	F3-6E_contig00272	blaTEM-1B	IS26	None	2,597
RGU	F5-4	ecoli	973	D	F5-4_contig00262	blaTEM-1B	IS26	None	2,368
RGU	165	ecoli	182	E	165_contig00028	aph(3*)-Ib, aph(6)-Id, blaTEM-1B, dfrA7, qacE, sul1, sul2	IS26, IS26, Tn6205	IncQ1	46,619
RGU	166	ecoli	182	E	166_contig00018	aph(3*)-Ib, aph(6)-Id, blaTEM-1B, dfrA7, qacE, sul1, sul2, tet(A)	IS26, IS26, IS26, Tn6205	IncQ1	71,698
SMU	ABPCE194	ecoli	648	F	ABPCE194_contig00016	aph(3*)-Ib, aph(6)-Id, blaTEM-1B, dfrA14, sul2	IS26, IS26, IS30, IS5075, ISEc37, Tn2, Tn6205	IncFIC(FII)	99,270
SMU	ABPCE195	ecoli	648	F	ABPCE195_contig00018	aph(3*)-Ib, aph(6)-Id, blaTEM-1B, dfrA14, sul2	IS26, IS26, IS30, IS5075, ISEc37, Tn2, Tn6205	IncFIA, IncFIC(FII)	97,908
SMU	ABPCE198	ecoli	648	F	ABPCE198_contig00017	aph(3*)-Ib, aph(6)-Id, blaTEM-1B, sul2	IS26, IS30, IS5075, ISEc37, Tn2, Tn6205	IncFIA, IncFIC(FII)	93,948
RGU	175	ecoli	N/A	G	175_contig00014	aph(3*)-Ib, aph(6)-Id, blaTEM-1B, dfrA5, sul2, tet(A)	IS26, IS26, Tn1721, Tn6205	IncQ1	23,742
RGU	S10-5E	fergusonii	7852	fergusonii	S10-5E_contig00137	aph(3*)-Ib, aph(6)-Id, blaTEM-1B, dfrA14, sul2	IS26, IS5075, Tn6205	None	11,277

Phylogenetic group

A

B1

B2

D

E

F

E. fergusonii

***bla*_{TEM-1} gene and its surrounding structure (IS26) are present in ampicillin resistant bacteria of livestock, aquatic, vegetable, and human clinical origin**

Conclusion

1. The frequency of the transmission of ARB and ARGs to crops via livestock waste low.
2. Contamination rate of *E. coli* in vegetable in Japan is very low
3. Strains harboring the *bla*TEM gene and its surrounding structure (IS26) were spreading among several sources

The possibility of transmission of livestock-derived ARB to humans via soil and vegetables cannot be ruled out, but the risk is extremely low in Japan.