

# Vancomycin resistant enterococci (VRE) isolates from human and animals

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The isolation of vancomycin resistant enterococci (VRE) was first reported in 1988 in the United Kingdom and France, and shortly thereafter, VRE were detected in hospitals in the United States. Since then, VRE have emerged with unanticipated rapidity and now encountered in most hospitals, especially in the United States. Drug resistant bacteria are selectively increased by increasing use of antimicrobial agents. A major factor that has contributed to the dissemination of VRE in the United States and Europe is now evident. In the United States, it is likely that the excessive use of glycopeptide antibiotics in the health care environment has resulted in the selective increase of VRE in the human intestine, which has subsequently spread by nosocomial transmission. In Europe, it is strongly suggested that the use of avoparcin as a growth promoter in animal feed has resulted in the selective increase of VRE in the human community. In both cases, the direct selective pressure of glycopeptides is the largest contributing factor in the selective increase of VRE in different habitats. Besides the direct selective pressure of antibiotics for increasing selectively in drug resistant bacteria, the genetic transfer system of an organism is essential to the spread of drug resistance in the organism.

VRE strains isolated from human (patients), animals and meats in Japan, Korea, and Taiwan were analyzed. In case of Japan, VRE strains have been isolated from imported chickens from the countries where avoparcin has been used in food animals. The most of VanA type VRE isolates from Thai chickens showed VanB type phenotype that exhibited high level vancomycin resistance and relatively low level teicoplanin

resistance, and had three amino acid substitutions in the VanS gene of VanA type determinants. The VRE strains showing the same characteristics as the VRE strains isolated from the Thai chickens were frequently isolated from patients in Japan. Eighty seven VRE strains, of which 56 were isolated from chicken feces and 31 were isolated from hospital patients in Korea, were examined in the drug resistance and the plasmid. The two plasmids, pSL1 of *E. faecalis* KV1 and pSL2 of *E. faecalis* KV2, which were isolated from patient and chicken feces, respectively, were the same pheromone responsive plasmid which encodes VCM, GM, KM, SM and EM resistances. In Taiwan, the pheromone responsive conjugative plasmids which encode VCM, EM and BC (bacitracin) resistances were frequently isolated from both animals and patients VanA type *E. faecalis* VRE. The VanA type strains showed the same characteristics as the VRE strains isolated from Thai chickens in Japan.

**Vancomycin resistant enterococcus (VRE)  
isolates from human and animals**

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## Topics

- Introduction of the VRE  
the mechanism of vancomycin resistance (VCM<sup>r</sup>) of enterococci
- VRE from human and animals in Japan
- VCM<sup>r</sup> plasmid from human and animal VRE in Korea
- VRE from human and animals in Taiwan

## Enterococci

- Gram positive cocci
- Normal resident flora in the animal and human intestines
- Cause opportunistic infection to human
- Abundant in genetic exchange system (conjugative plasmid, transposon, phage)

**Vancomycin resistant enterococci (VRE)  
Glycopeptide resistant enterococci (GRE)**

- Acquisition of specific resistance genes (*van* genes)
- Multiple drug resistance
- *E. faecium* (80~90%), *E. faecalis*
- Limited options for treatment of VRE infection
- First isolation in UK and France (1988)
- Rapid dissemination

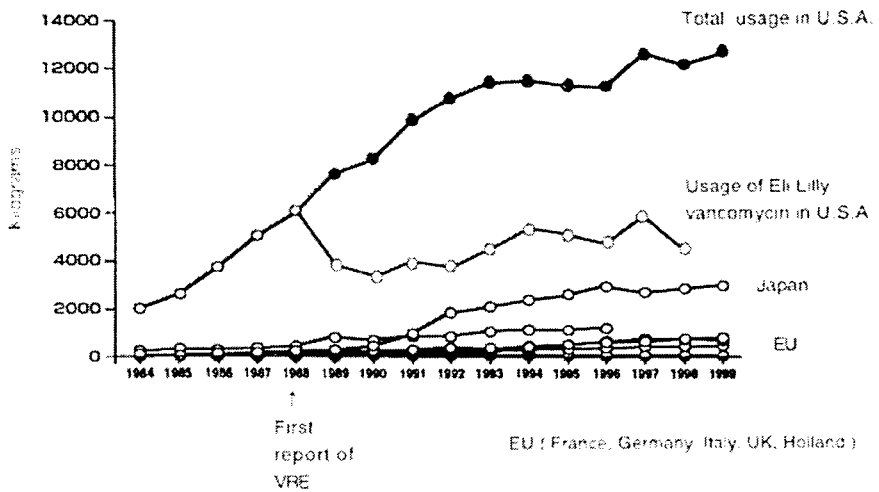
**Emergence and dissemination of  
drug resistant bacteria (VRE)**

- Selection by use of the antibiotics
- Horizontal gene transfer by genetic exchange systems  
(acquired drug resistances:  
vancomycin resistance genes)

## Usage of glycopeptides and increase of the vancomycin resistant enterococci

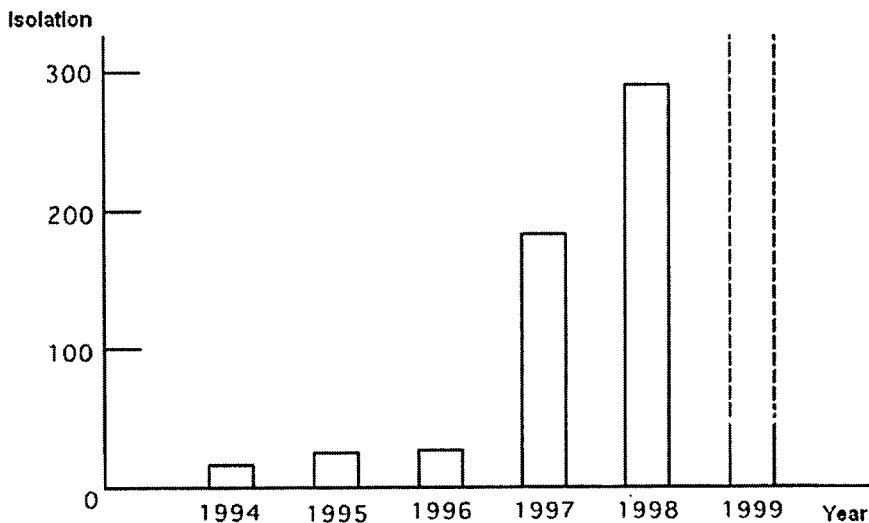
Country	Dissemination of VRE	Use of drug	
		Glycopeptides	Purpose
EU countries	Environment (Animals)	Avoparcin	Feeding
U.S.A.	Hospitals (Human)	Vancomycin (VCM) Teicoplanin (TEIC)	Medical

### **Usage of vancomycin in the United States, EU and Japan**

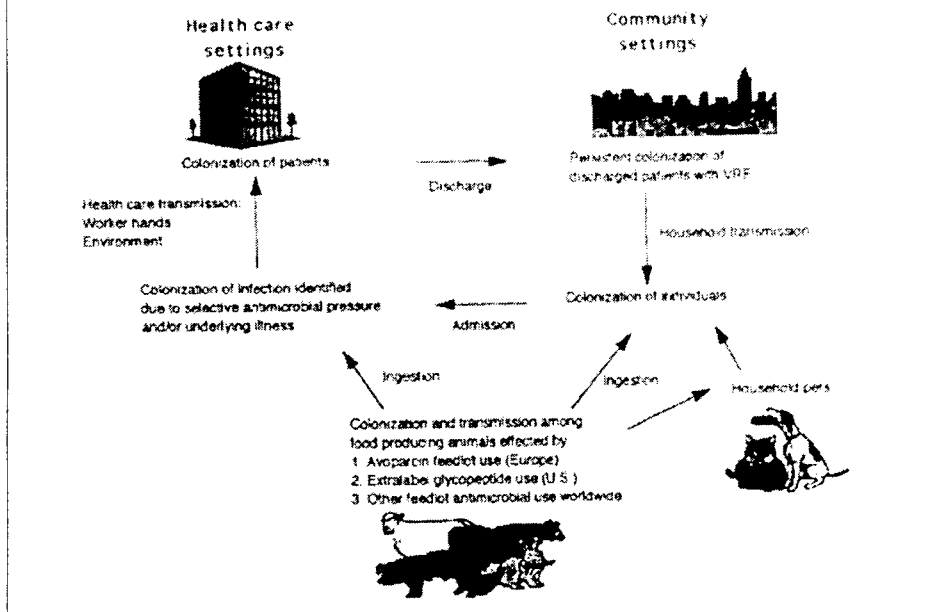


(Adapted from A. Agron & C. Chaudron, J. Med. Microbiol. 45 (1998) 1-10)

## Isolation of VRE strains in the Michigan Medical School hospital (US)



## Potential interaction between community and health-care settings in the transmission of vancomycin resistant enterococci (VRE)



## Ban of avoparcin and decrease of VRE isolation in animals in Netherland

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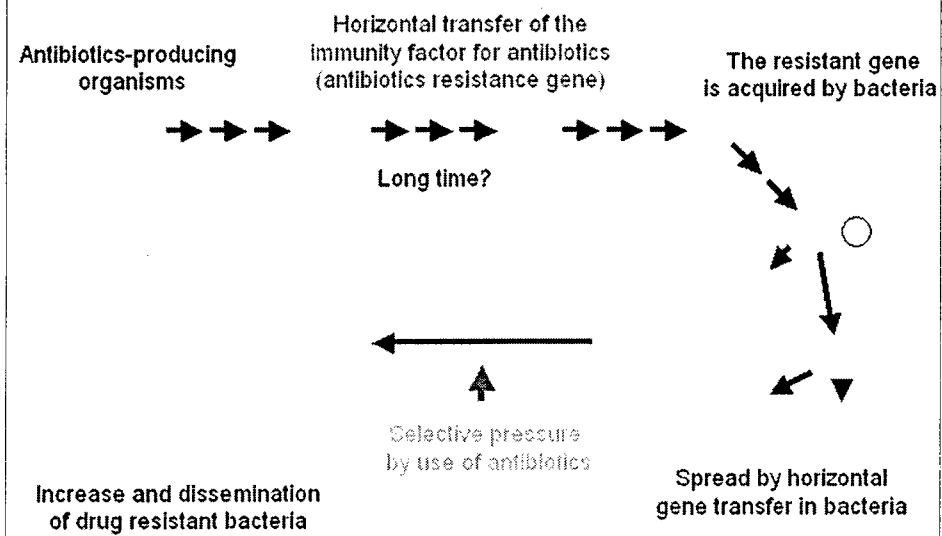
### Emergence and dissemination of drug resistant bacteria

- Selection by usage of antibiotics
- Horizontal gene transfer by genetic exchange systems  
(acquired drug resistances; vancomycin resistance)

## Horizontal gene transfer by genetic exchange systems in bacteria

- Transformation
- Transduction by phage
- Conjugation by the conjugative plasmid

## Origin of the drug resistance gene and spread of the resistance gene

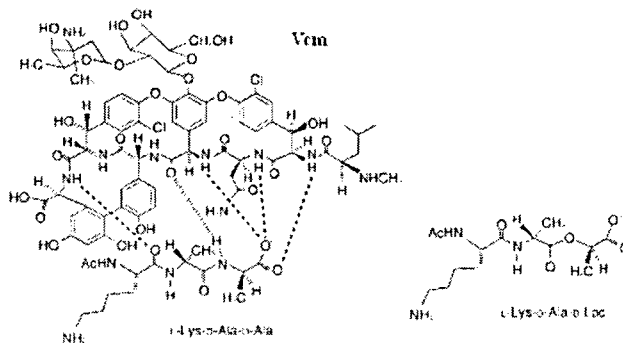




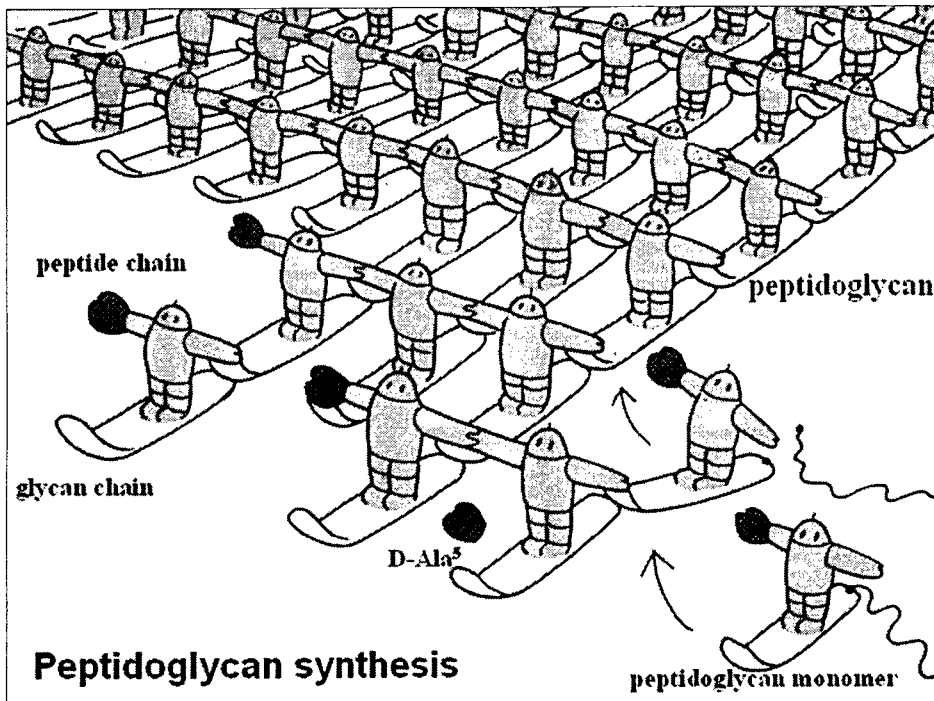
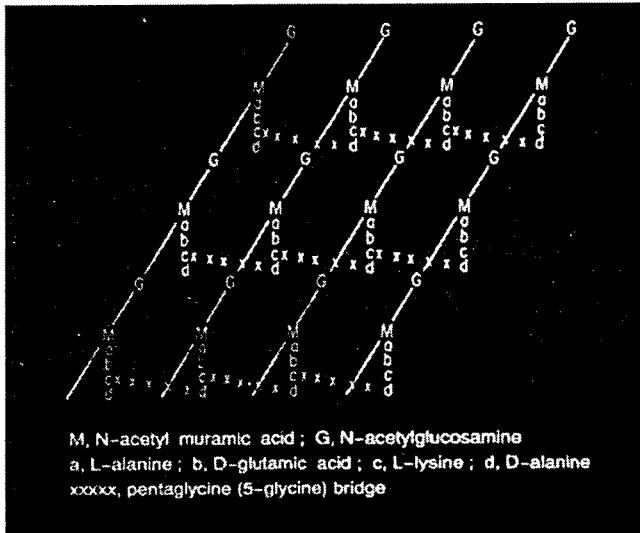
## The mechanism of vancomycin resistance in enterococci

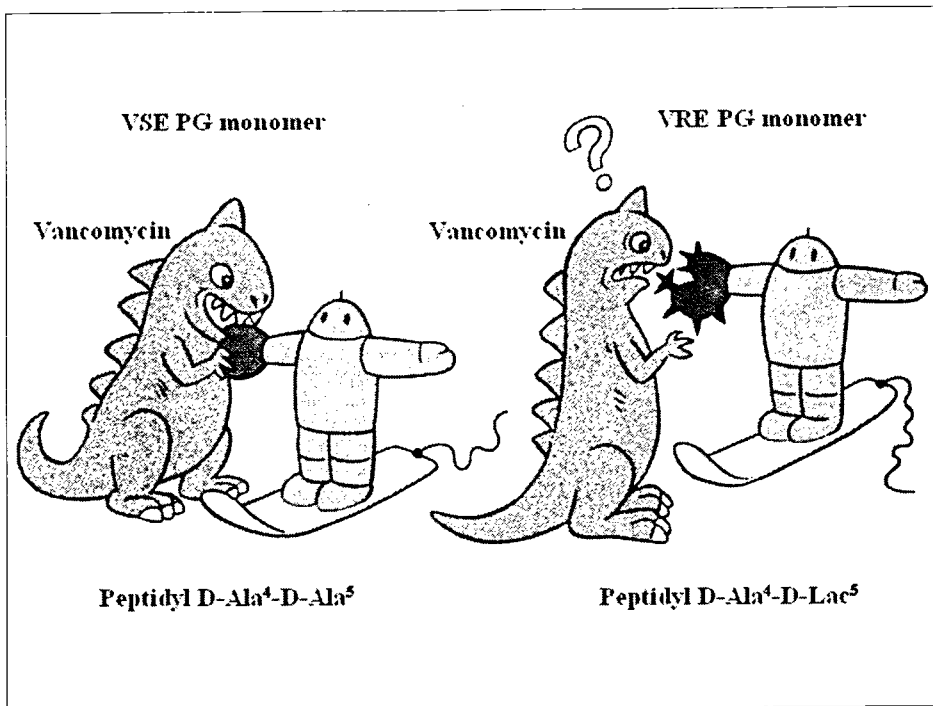
### Glycopeptide

- Vancomycin (VCM), Teicoplanin (TEIC), Avoparcin
- Inhibition of peptidoglycan (cell wall) synthesis
- Use to treat MRSA infection (used for feeding)



## Structure of cell wall peptidoglycan of Gram-positive bacteria

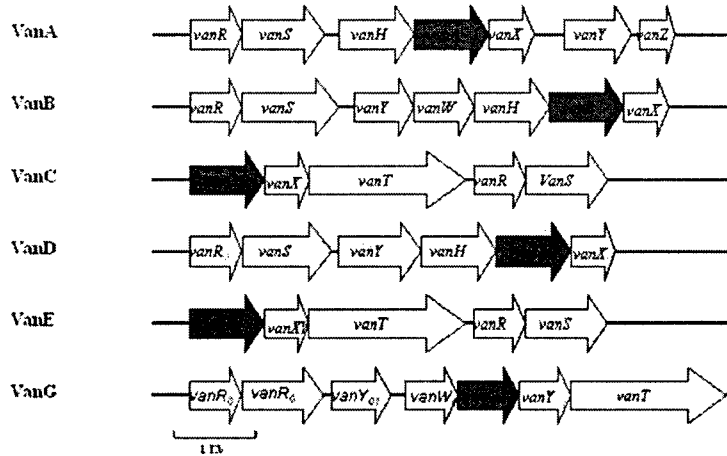




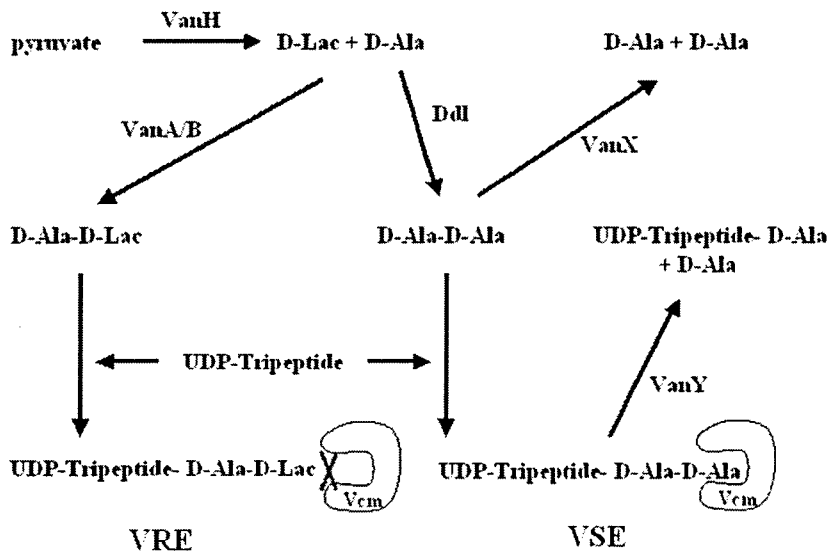
### Type of vancomycin resistance gene (genotype)

Genotype	VCM MIC (mg·L)	TEIC MIC (mg·L)	Location	Expression	Precursor	Species
<i>vanA</i>	64-1000	16-512	Plasmid, Chromosome	Inducible	D-Ala-D-Lac	<i>E. faecalis</i> , <i>E. faecium</i> , etc
<i>vanB</i>	4-1000	≤ 1	Plasmid, Chromosome	Inducible	D-Ala-D-Lac	<i>E. faecalis</i> , <i>E. faecium</i>
<i>vanC</i>	2-32	≤ 1	Chromosome	Constitutive, Inducible	D-Ala-D-Ser	<i>E. casseliflavus</i> , <i>E. gallinarum</i> , <i>E. flavescens</i>
<i>vanD</i>	64-128	4-64	Chromosome	Constitutive	D-Ala-D-Lac	<i>E. faecium</i> <i>E. raffinosus</i>
<i>vanE</i>	16	0.5	?	Inducible	D-Ala-D-Ser	<i>E. faecalis</i>
<i>vanG</i>	<16	<0.5	?	?	?	<i>E. faecalis</i>

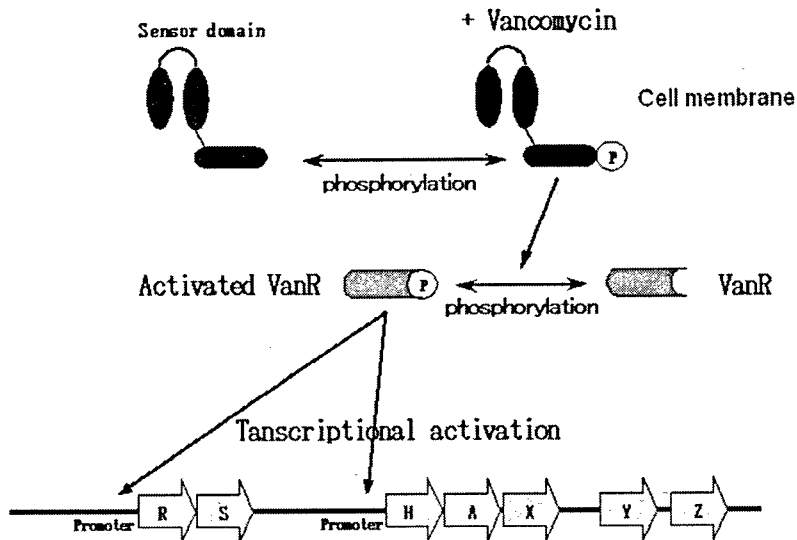
## Vancomycin resistance operons



## Peptidoglycan synthesis pathway of VRE and VSE



## Expression of vancomycin resistance: two component system

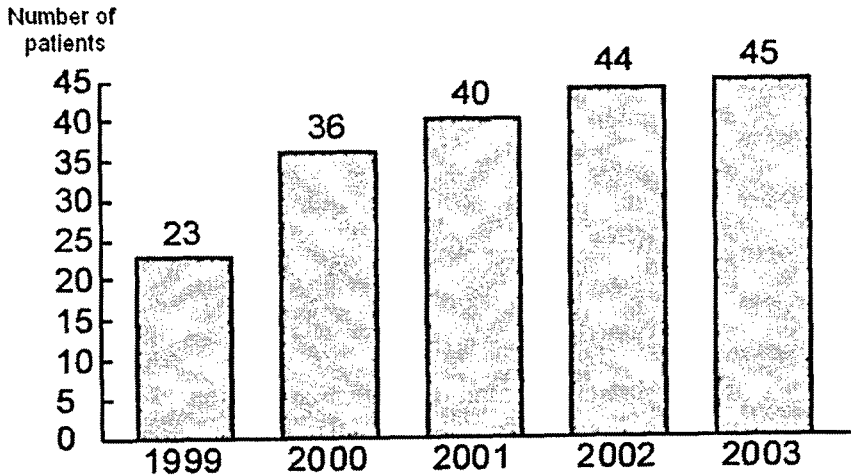


## Analysis of VRE strains isolated from animals and human in Japan

The VanA-type VRE with low MIC to  
TEI were isolated from the imported  
chicken meats and human

## Isolation of VRE from patients (human) in Japan

The isolation of VRE is increasing but not so much in Japan



## Isolation of VanA-genotype VRE from imported chicken meats in Japan

- VanA-type VRE strains were isolated from the imported chicken meats.
- The animals were from the countries where the avoparcin have been used for feeding.
- Most of the VanA-type VRE strains (11/11; 100%) from Thailand's chicken meats showed the low MIC to TEI and had VanS mutantions.

## Isolation of VanA-type VRE from imported chicken meats in Japan (1998-1999)

Countries	Use of Avoparcin	Samples	Isolation
Thailand	Yes	57	12/57 (21 %)
France	Yes	10	5/10 (50 %)
Brazil	Yes	56	2/56 ( 4 %)
U.S.A.	No	51	0/51 (0 %)
China	No	71	0/71 (0 %)
Vietnam	No	3	0/3 (0 %)

## VRE isolated from humans and imported chickens in Japan

Clon and species	Strain <sup>a</sup>	Origin	Source (specimen and/or reference)	Transfer frequency of vancomycin resistance from wild-type strain to <i>E. faecalis</i> FA22 (no. of transconjugants/donor cell)	MIC (µg/ml)		Deleted amino acid sequence of vanA and substitution no. of VanA-type determinant
					Vancomycin	Ticoplanin	
<b>High tetracycline resistance</b>							
<i>E. faecalis</i>	BM4147	France	Human (H)	<10 <sup>-8</sup>	1/24	256	Prototype
<i>E. faecalis</i>	TV1	Japan	Human (feces) (9)	<10 <sup>-8</sup>	288	128	Prototype
<i>E. faecalis</i>	TV3	Japan	Human (feces)	<10 <sup>-8</sup>	512	128	Prototype
<i>E. faecalis</i>	TV2	Japan	Human (feces)	<10 <sup>-8</sup>	512	64	Prototype
<i>E. faecalis</i>	TV3	Japan	Human (drainage)	<10 <sup>-8</sup>	512	64	Prototype
<i>E. faecalis</i>	TV4	Japan	Human (feces)	<10 <sup>-8</sup>	512	64	Prototype
<i>E. faecalis</i>	TV1	France	Chicken	<10 <sup>-8</sup>	512	64	Prototype
<i>E. faecalis</i>	TV2	France	Chicken	<10 <sup>-8</sup>	512	64	Prototype
<i>E. faecalis</i>	TV3	France	Chicken	<10 <sup>-8</sup>	512	128	TS41
<b>Low tetracycline resistance</b>							
<i>E. faecalis</i>	KV12	Japan	Human (feces)	10 <sup>-7</sup>	1/24	4	LSW, LSQ, Q641
<i>E. faecalis</i>	KV21	Japan	Human (feces)	10 <sup>-7</sup>	512	2	LSW, LSQ, Q641
<i>E. faecalis</i>	CV1	Japan	Human (feces)	<10 <sup>-8</sup>	512	0.8	LSW, LSQ, Q641
<i>E. faecalis</i>	TV1	Ireland	Chicken	10 <sup>-7</sup>	512	4	LSW, LSQ, Q641
<i>E. faecalis</i>	TV2	Ireland	Chicken	<10 <sup>-8</sup>	512	4	LSW, LSQ, Q641
<i>E. faecalis</i>	TV3	Ireland	Chicken	<10 <sup>-8</sup>	512	16	LSW, LSQ, Q641
<i>E. faecalis</i>	TV4	Ireland	Chicken	<10 <sup>-8</sup>	1/24	16	LSW, LSQ, Q641
<i>E. faecalis</i>	TV5	Ireland	Chicken	10 <sup>-7</sup>	1/24	2	LSW, LSQ, Q641
<i>E. faecalis</i>	TV6	Ireland	Chicken	<10 <sup>-8</sup>	256	4	LSW, LSQ, Q641
<i>E. faecalis</i>	TV7	Ireland	Chicken	<10 <sup>-8</sup>	512	2	LSW, LSQ, Q641
<i>E. faecalis</i>	TV8	Ireland	Chicken	<10 <sup>-8</sup>	512	2	LSW, LSQ, Q641
<i>E. faecalis</i>	TV9	Ireland	Chicken	10 <sup>-7</sup>	512	4	LSW, LSQ, Q641
<i>E. faecalis</i>	TV10	Ireland	Chicken	10 <sup>-7</sup>	512	16	LSW, LSQ, Q641
<i>E. faecalis</i>	TV11	Ireland	Chicken	10 <sup>-7</sup>	1/24	16	LSW, LSQ, Q641
<i>E. faecalis</i>	GV2	Japan	Broiler farm droppings (15)	10 <sup>-7</sup>	512	2	LSW, LSQ, Q641

## Operon structure of the VanA-type resistance genes

### Prototype VanA operon (*E. faecium* BM4147)



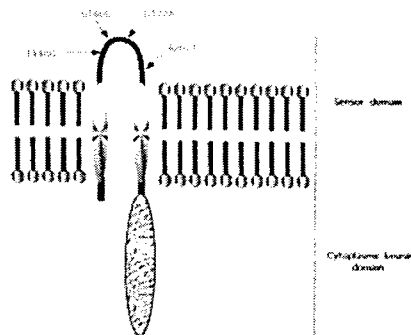
### VanA genotype with VanB phenotype operon (Thailand type)



3 amino acids mutation in VanS  
L50V, E54Q, Q69H

## VanS structure of the VanA-type (Thailand-type) VRE showing low MIC to TEIC

1) Wild type or prototype	sensor domain			cytoplasmic kinase domain
nucleotide number (amino acid)	148 (E)	160 (E)	177 (D)	297 (A) 1152 (E)
2) Mutates of one nucleotide				A (N)
3) Mutation of three nucleotides	G (V)	G (V)	G (V)	





## VanA-type VRE sensitive to teicoplanin

- VanA-type VRE usually shows high-resistance (high MIC) to both VCM and TEI.
- VanA-type VRE with low MIC of TEI (similar to VanB-phenotype) caused by VanS mutations were isolated from Thailand's chicken meats.

## Isolation of VRE in Japan (a survey in 2002)

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Sending a questionnaire 4,426

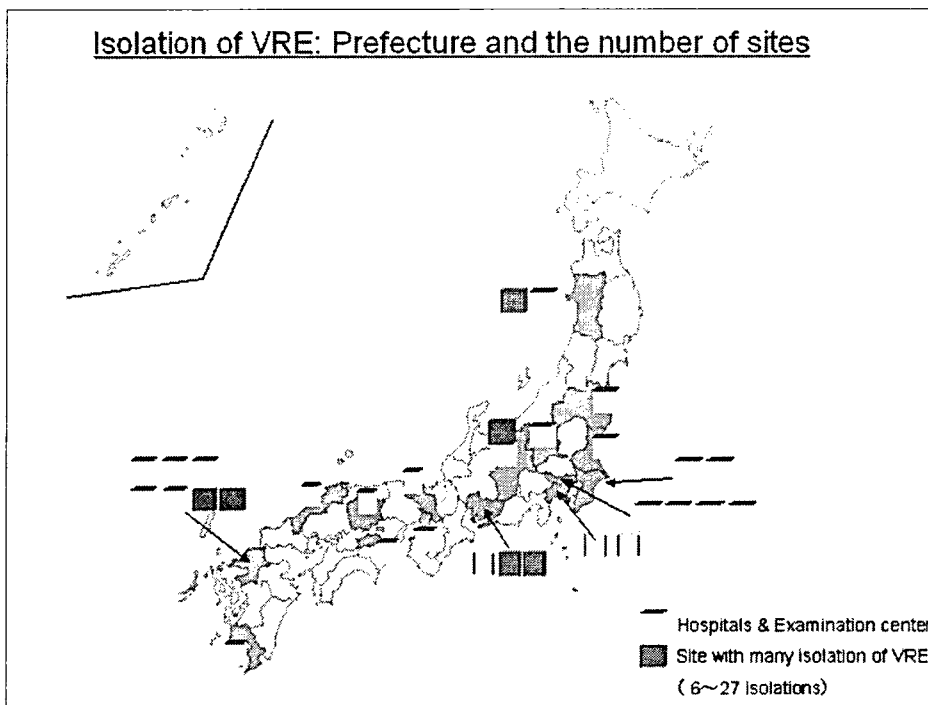
[ 3,882 hospitals (19 ~1,500 beds)  
544 Examination center (private)

Percentage of answer 1,778 (40.2 %)

Isolation of VRE 31 sites (1.7 %)

[ 26 hospitals  
5 Examination center

### Isolation of VRE: Prefecture and the number of sites



### **Type of VRE isolated**

VanA	14 sites (12 hospitals+2)
VanB	15 sites (12 hospitals+3)
VanA & VanB	1 site (1 hospital)
VanD	1 site (1 hospital)
<b>total</b>	<b>31 sites (26 hospitals+5)</b>

### **Type of VRE isolated from human**

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VanA	71	(55 %)
VanB	56	(44 %)
VanD	1	(1 %)

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total	128	(100 %)
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### **Species of VRE**

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<i>E. faecalis</i>	41	(48 %)
<i>E. faecium</i>	41	(48 %)
<i>E. avium</i>	3	(3.5%)
<i>E. raffinosus</i>	1	(1.1%)

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total	86	(100 %)
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### Analysis of the VanS mutations of VanA-type VRE from patients in Japan

Case	Hospitals	Species	Type (PCR)	MIC ( $\mu\text{g/ml}$ )		Mutations of VanS
				VCM	TEIG	
16	IKL	<i>E. faecium</i>	VanA	512	64	Prototype
20	TTH	<i>E. faecium</i>	VanA	256	32	Prototype
21		<i>E. faecium</i>	VanA	128	1	L50V, E54Q, Q69H
		<i>E. avium</i>	VanA	256	0.5	L50V, E54Q, Q70H
24	CRH	<i>E. faecium</i>	VanA	1024	128	Prototype
25		<i>E. faecium</i>	VanA	1024	128	Prototype
55	KRH	<i>E. faecium</i>	VanA	1024	64	Prototype
56		<i>E. faecium</i>	VanA	1024	32	Prototype
57		<i>E. faecium</i>	VanA	1024	128	Prototype
58		<i>E. faecium</i>	VanA	1024	256	Prototype
59		<i>E. faecium</i>	VanA	1024	32	Prototype
60		<i>E. faecium</i>	VanA	1024	128	Prototype
61	OMH	<i>E. faecium</i>	VanA	1024	32	Prototype
75-1		<i>E. faecalis</i>	VanA	512	4	L50V, E54Q, Q69H
75-2		<i>E. faecalis</i>	VanA	512	4	L50V, E54Q, Q69H
75-3		<i>E. faecalis</i>	VanA	512	2	L50V, E54Q, Q69H
78-1	ONH	<i>E. faecium</i>	VanA	512	32	Prototype
78-2		<i>E. faecium</i>	VanA	256	8	Prototype
79	SUH	<i>E. faecalis</i>	VanA	1024	8	L50V, E54Q, Q69H
115	FYH	<i>E. faecalis</i>	VanA	1024	256	Prototype
116		<i>E. faecalis</i>	VanA	1024	256	Prototype
121	KCL	<i>E. faecium</i>	VanA	1024	1	L50V, E54Q, Q69H
122		<i>E. faecalis</i>	VanA	1024	256	L50V, E54Q, Q69H
2188	Thailand VRE	<i>E. faecalis</i>	VanA	512	4	L50V, E54Q, Q69H

L, leucine; V, valine; E, glutamic acid; Q, glutamine; H, histidine

### Features of the VRE from patients (human) in Japan

- VanB-type VREs have been isolated more frequently (44%) comparing to US and Europe.
- *E. faecalis* has been isolated more frequently (48%) comparing to US and Europe.
- VanA-type VRE with low MIC of TEI that had VanS-mutations identical to the Thailand's chicken VRE were isolated frequently (about 30%)

## Analysis of VRE isolated from human and animals in Japan

- Most of the VanA-type VRE isolated from the imported Thailand's chicken meats showed the low MIC of TEI caused by VanS-mutations (Thailand-type VRE)
- The same VCM<sup>r</sup> genes were disseminated in VRE strains from patients (human)

## Analysis of the vancomycin resistant conjugative plasmid isolated from human and animal in Korea

The same vancomycin resistant  
conjugative plasmid in both  
human and animal

## VRE strains from Korea

- 87 VRE strains isolated in Korea

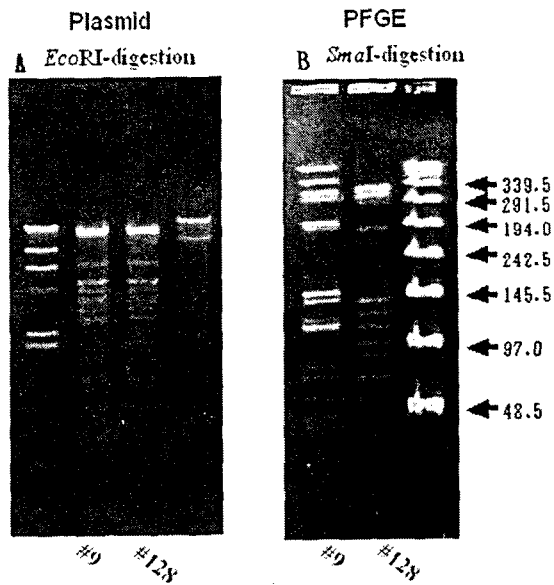
56 strains from chicken feces

31 strains from patients

### Two VanA-type VRE (#9, #128) from different samples had the same plasmid.

- Each of #9 VRE from a patient and #128 VRE from chicken feces had vancomycin resistant plasmid with the same restriction fragment profile.
- Both VRE strains were VanA-type *E. faecalis*.
- Both VCM<sup>r</sup> plasmids were conjugative.
- Both plasmids had the same drug resistances.  
VCM<sup>r</sup>, GM<sup>r</sup>, KM<sup>r</sup>, SM<sup>r</sup>, EM<sup>r</sup>

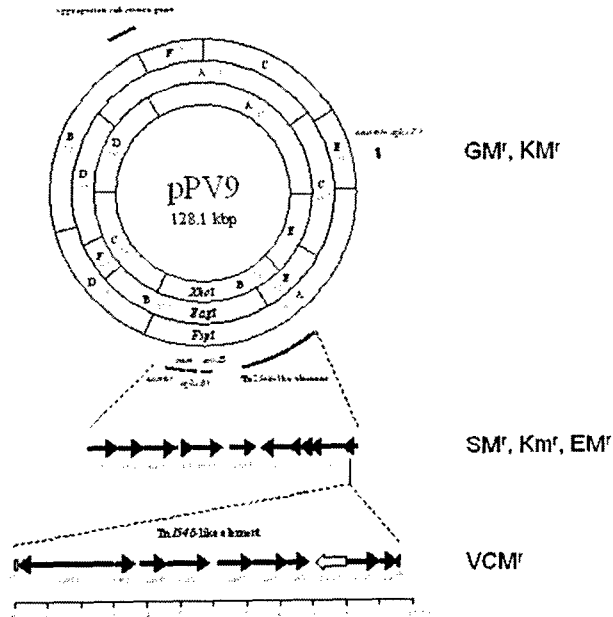
Plasmid and PFGE profile of the #9 and #128 VRE



The same VCM<sup>r</sup> plasmid in different VRE

The drug resistant conjugative plasmid pPV9  
(VCM<sup>r</sup>, GM<sup>r</sup>, KM<sup>r</sup>, SM<sup>r</sup>, EM<sup>r</sup>)

## Structure of the multiple drug resistant plasmid pPV9



## Analysis of the VCM<sup>r</sup> plasmid from Korean VRE

- The multiple drug resistant conjugative plasmid might transmitted between human and animals.



# Analysis of the VRE strains isolated from human and animals in Taiwan

The dissemination of the same VanA-type (Thailand-type) VCM<sup>r</sup> conjugative plasmids among human and animals in Taiwan

**VanA type Taiwan's VRE isolated from Human (40 strains)**

Strain	Origin	Species	MIC (µg/ml)		Drug resistances	Strain	Species	MIC (µg/ml)		Drug resistances
			Van	Tei				Van	Tei	
TRH01	Animals	<i>E. faecalis</i>	<100	1	van ER Tet CM F D SM Cps	TRH49	<i>E. faecalis</i>	>100	64	van Tet ER Tet CM F D SM Cps
TRH02	Wound	<i>E. faecalis</i>	>100	4.5	van ER Tet CM F D Cps	TRH50	<i>E. faecalis</i>	>100	64	van Tet ER Tet CM F D Cps
TRH03	Wound	<i>E. faecalis</i>	>100	4.5	van ER Tet CM F D Cps	TRH51	<i>E. faecalis</i>	>100	64	van Tet ER Tet CM F D Cps
TRH04	Urine	<i>E. faecalis</i>	>100	4.5	van ER Tet CM F D Cps	TRH52	<i>E. faecalis</i>	>100	64	van Tet ER Tet CM F D Cps
TRH05	Rat	<i>E. faecalis</i>	>100	4.5	van ER Tet CM F D SM Cps	TRH53	<i>E. faecalis</i>	>100	64	van Tet ER Tet CM F D Cps
TRH06	Wound	<i>E. faecalis</i>	>100	0.25	van ER Tet CM F D SM Cps	TRH54	<i>E. faecalis</i>	>100	64	van Tet ER Tet CM F D Cps
TRH07	Wound	<i>E. faecalis</i>	>100	2	van ER Tet CM F D Cps	TRH55	<i>E. faecalis</i>	>100	64	van Tet ER Tet CM F D SM Cps
TRH08	Wound	<i>E. faecalis</i>	>100	6.5	van ER Tet CM F D Cps	TRH56	<i>E. faecalis</i>	>100	64	van Tet ER Tet CM F D SM Cps
TRH09	Blood	<i>E. faecalis</i>	>100	6.5	van ER Tet CM F D SM Cps	TRH57	<i>E. faecalis</i>	>100	64	van Tet ER Tet CM F D SM Cps
TRH10	Blood	<i>E. faecalis</i>	>100	1	van ER Tet F D Cps	TRH58	<i>E. faecalis</i>	>100	64	van Tet ER Tet CM F D SM Cps
TRH11	Wound	<i>E. faecalis</i>	>100	2	van ER Tet CM F D SM Cps	TRH59	<i>E. faecalis</i>	>100	64	van Tet ER Tet CM F D SM Cps
TRH12	Wound	<i>E. faecalis</i>	>100	4	van ER Tet F D Cps	TRH60	<i>E. faecalis</i>	>100	256	van Tet ER Tet CM Cps
TRH13	Wound	<i>E. faecalis</i>	>100	2	van ER Tet CM F D SM Cps					
TRH14	Wound	<i>E. faecalis</i>	>100	1.5	van ER Tet F D Cps					
TRH15	Wound	<i>E. faecalis</i>	>100	4.5	van ER Tet F D SM Cps					
TRH16	Wound	<i>E. faecalis</i>	>100	4.5	van ER Tet CM F D SM Cps					
TRH17	Abcess	<i>E. faecalis</i>	>100	8.5	van ER Tet CM F D SM					
TRH18	Wound	<i>E. faecalis</i>	>100	6.5	van ER Tet F D SM					
TRH19	Wound	<i>E. faecalis</i>	>100	2	van ER Tet CM F D SM Cps					
TRH20	Animals	<i>E. faecalis</i>	>100	4.5	van ER Tet F D SM					
TRH21	Wound	<i>E. faecalis</i>	>100	4.5	van ER Tet CM F D SM Cps					
TRH22	Wound	<i>E. faecalis</i>	>100	6.5	van ER Tet Tet					
TRH23	Blood	<i>E. faecalis</i>	>100	2	van ER Tet					
TRH24	Burpna	<i>E. faecalis</i>	>100	4	van ER Tet CM F D SM Cps Cps					
TRH25	Urine	<i>E. faecalis</i>	>100	2	van ER Tet Cps					
TRH26	Drain	<i>E. faecalis</i>	>100	2	van ER Tet F D SM Cps					
TRH27		<i>E. faecalis</i>	>100	4	van ER Tet F D SM Cps					
TRH28		<i>E. faecalis</i>	>100	2	van ER Tet Cps					

### VanA type Taiwan's VRE isolated from animals (30 strains)

Strain	Origin	Species	MIC ( $\mu\text{g/ml}$ )		Drug resistances
			Van	Tel	
TVA 01	Chicken meat	<i>E. faecalis</i>	>256	4	Van EEE Tet C Clm F III SII Spe
TVA 02	Chicken meat	<i>E. faecalis</i>	>256	2	Van EEE Tet C Clm III
TVA 03	Chicken meat	<i>E. faecalis</i>	>256	4	Van EEE Tet C Clm F III SII Spe
TVA 04	Chicken meat	<i>E. faecalis</i>	>256	1	Van EEE Tet III
TVA 05	Chicken meat	<i>E. faecalis</i>	>256	1	Van EEE Tet C Clm F III SII Spe
TVA 06	Chicken meat	<i>E. faecalis</i>	>256	3	Van EEE Tet C Clm F III SII
TVA 07	Chicken meat	<i>E. faecalis</i>	>256	8	Van EEE Tet C Clm F III SII Spe
TVA 08	Chicken meat	<i>E. faecalis</i>	>256	2	Van EEE Tet C Clm F III SII Spe
TVA 09	Chicken meat	<i>E. faecalis</i>	>256	4	Van EEE Tet C Clm F III SII Spe
TVA 10	Chicken meat	<i>E. faecalis</i>	>256	4	Van EEE Tet C Clm F III SII Spe
TVA 11	Chicken meat	<i>E. faecalis</i>	>256	8	Van EEE Tet C Clm F III SII Spe
TVA 12	Chicken meat	<i>E. faecalis</i>	>256	2	Van EEE Tet C Clm F III SII Spe
TVA 13	Chicken meat	<i>E. faecalis</i>	>256	8	Van EEE Tet C Clm
TVA 14	Pig waste	<i>E. faecalis</i>	>256	2	Van EEE Tet C Clm F III SII Spe
TVA 15	Pig waste	<i>E. faecalis</i>	>256	8	Van EEE Tet C Clm III
TVA 16	Pig waste	<i>E. faecalis</i>	>256	8	Van EEE Tet C Clm F III SII Spe Cpt
TVA 17	Pig waste	<i>E. faecalis</i>	>256	4	Van EEE Tet C Clm F III SII Spe
TVA 18	Pig waste	<i>E. faecalis</i>	>256	8	Van EEE Tet C Clm Cpt
TVA 19	Pig waste	<i>E. faecalis</i>	>256	8	Van EEE Tet C Clm F III SII Cpt
TVA 20	Pig waste	<i>E. faecalis</i>	>256	4	Van EEE Tet C Clm F III SII Cpt
TVA 21	Chicken feather	<i>E. faecalis</i>	>256	4	Van EEE Tet C Clm F III SII Cpt
TVA 22	Chicken feather	<i>E. faecalis</i>	>256	8	Van EEE Tet C Clm F III SII Spe
TVA 23	Chicken feather	<i>E. faecalis</i>	>256	2	Van EEE Tet III SII
TVA 24	Chicken feather	<i>E. faecalis</i>	>256	2	Van Tet C Clm F III SII Cpt
TVA 25	Chicken feather	<i>E. faecalis</i>	>256	8,8	Van Tet C Clm F III SII Spe Cpt
TVA 26	Chicken feather	<i>E. faecalis</i>	>256	1	Van Tet C Clm F III SII Cpt
TVA 27	Chicken feather	<i>E. faecalis</i>	>256	1	Van Tet C Clm F III SII Cpt
TVA 28	Chicken feather	<i>E. faecalis</i>	>256	1	Van Tet C Clm F III SII Cpt
TVA 29	Chicken feather	<i>E. faecalis</i>	>256	8	Van Tet C Clm F III SII Cpt
TVA 30	Chicken feather	<i>E. faecalis</i>	>256	1	Van Tet C Clm F III Cpt

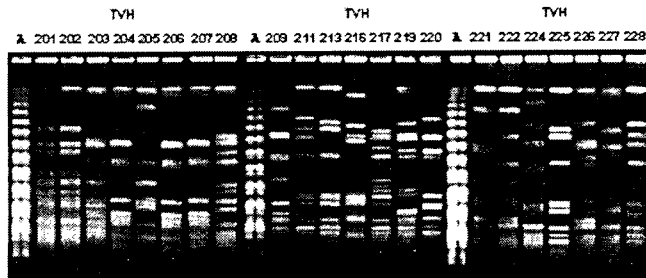
### Taiwan's VanA genotype VRE isolated from human (40 strains)

Species	Van phenotype	Strain (%)
<i>E. faecalis</i>	VanA (Van Tel)	1/40 (2.5%)
	VanB (Van)	22/40 (55.0%)
<i>E. faecium</i>	VanA (Van Tel)	11/40 (27.5%)
	VanB (Van)	6/40 (15.0%)

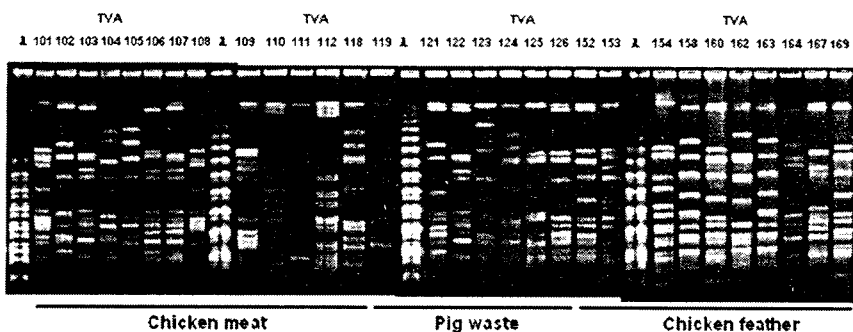
Taiwan's VanA genotype VRE isolated from animals (30 strains)

Species	Van Type	Isolates(%)
<i>E. faecalis</i>	VanB (Van)	30/30 (100%)

VanA genotype with VanB phenotype VRE isolated from human  
Pulsed Field agarose Gel Electrophoresis (PFGE)



VanA genotype with VanB phenotype VRE from animals  
Pulsed Field agarose Gel Electrophoresis (PFGE)



Transfer of the vancomycin resistance of VanA type  
VRE (*E. faecalis*) isolated from human

Strain	Transferability(%)	Transfer frequencies (per donor in broth mating)	
		Wild type → <i>E. faecalis</i> FA2-2	FA2-2 → <i>E. faecalis</i> JH2SS
23	22/23 (95.7%)	$10^{-5} \sim 10^{-3}$	$10^{-4} \sim 10^{-3}$

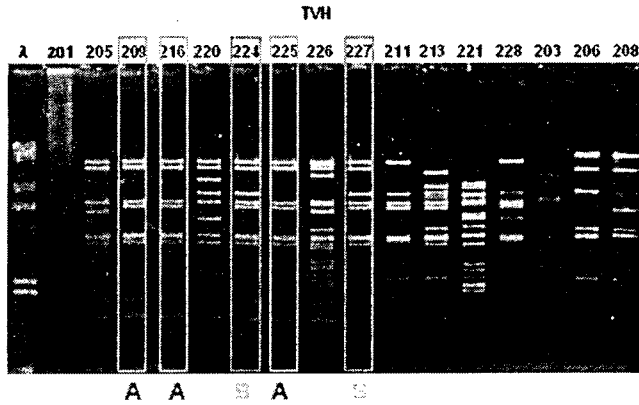
**Transfer of the vancomycin resistance of VanA type  
VRE (*E. faecium*) isolated from human**

Strain	Transferability(%)	Transfer frequencies (per donor in filter mating)	
		Wild type → <i>E. faecium</i> BMRF	FA2-2 → <i>E. faecium</i> BMSS
17	0/17 (0%)	<10 <sup>-7</sup>	<10 <sup>-7</sup>

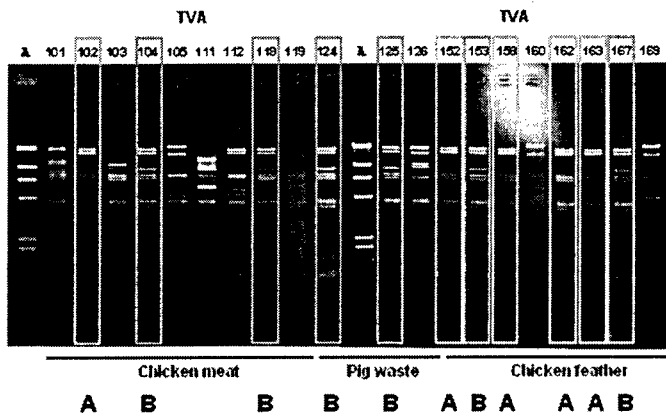
**Transfer of the vancomycin resistance of VanA type  
VRE (*E. faecalis*) isolated from animals**

Strain	Transferability (%)	Transfer frequencies (per donor in broth mating)	
		Wild type → <i>E. faecalis</i> FA2-2	FA2-2 → <i>E. faecalis</i> JH2SS
30	20/30 (66.7%)	10 <sup>-5</sup> ~10 <sup>-3</sup>	10 <sup>-4</sup> ~10 <sup>-3</sup>

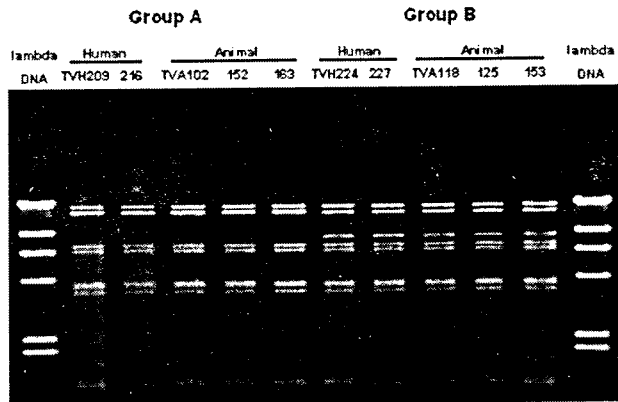
**Agarose gel electrophoresis (*Eco*RI digestion) of the vancomycin resistant conjugative plasmids isolated from human VanA type VRE (*E. faecalis*)**



**Agarose gel electrophoresis (*Eco*RI digestion) of the vancomycin resistant conjugative plasmids isolated from animal VanA type VRE (*E. faecalis*)**



**Comparison of the VanA type vancomycin resistant conjugative plasmids Isolated from animals and human VRE (*E. faecalis*) (EcoRI-digested plasmids on the agarose gel electrophoresis)**



**Operon structure of the VanA type resistance genes**

**Prototype VanA operon (*E. faecium* BM4147)**



**VanA genotype with VanB phenotype operon (Thailand type)**



3 amino acids mutation in VanS  
L50V, E54Q, Q69H

Two vancomycin resistant (Thailand type VanA)  
conjugative plasmids isolated from animals and  
human VRE (*E. faecalis*) in Taiwan

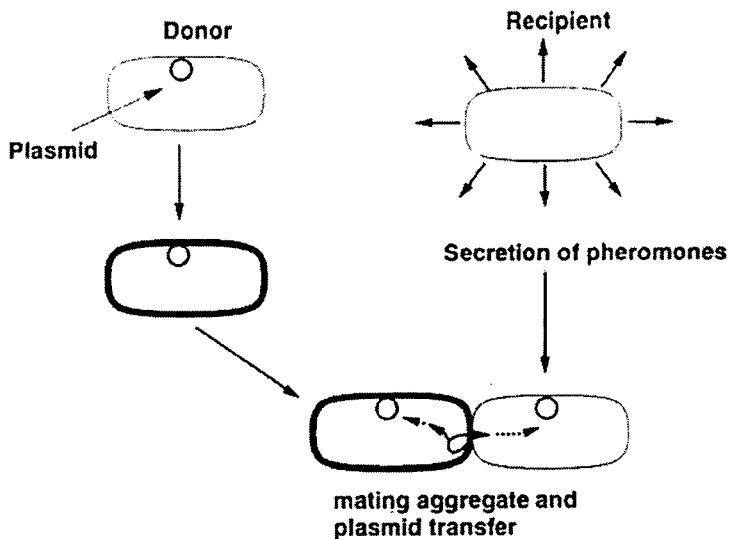
Group	Strain	Origin	VCM	TEC	EM	VanS mutations
A	TV#20	Human	512	8	256<	L50V, E54Q, Q69H
	TV#21	Human	512	8	256<	L50V, E54Q, Q69H
	TV#10	Animal	512	4	256<	L50V, E54Q, Q69H
	TV#12	Animal	512	4	256<	L50V, E54Q, Q69H
	TV#18	Animal	512	4	256	L50V, E54Q, Q69H
	TV#16	Animal	512	4	256<	L50V, E54Q, Q69H
	TV#13	Animal	512	4	256<	L50V, E54Q, Q69H
	TV#24	Human	512	8	256	L50V, E54Q, Q69H
	TV#27	Human	512	8	256	L50V, E54Q, Q69H
	B	TV#104	Animal	512	4	256
	TV#119	Animal	512	4	256	L50V, E54Q, Q69H
	TV#124	Animal	512	4	256	L50V, E54Q, Q69H
	TV#125	Animal	512	4	256<	L50V, E54Q, Q69H
	TV#157	Animal	512	4	256<	L50V, E54Q, Q69H

## Pheromone-responsive plasmids which transferred at a high frequency

(pheromones; cAD1, cOB1, cPD1, cCF10, cAM373)



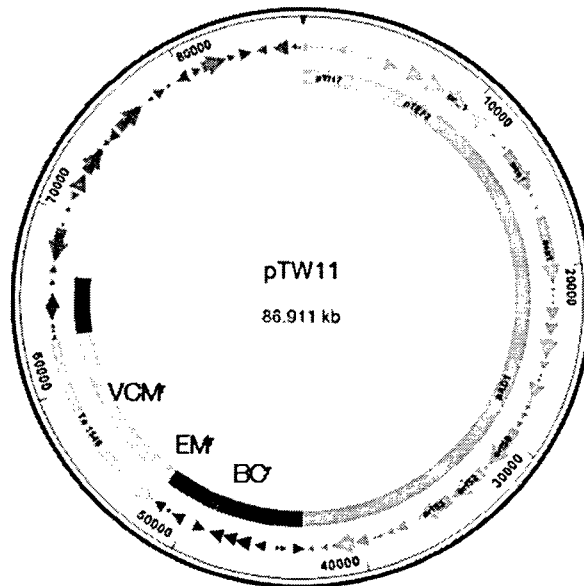
**Secretion of pheromones from *E. faecalis* recipient strains and the response of *E. faecalis* donor strains to the pheromone**



**Pheromone responsive conjugative VCM<sup>+</sup> plasmids from animals and human (cAD1, cOB1, cPD1, cCF10, cAM373)**

Animals		Human	
Strains	Pheromone	Strains	Pheromone
TVA101	other	TVH201	cAD1
TVA102	cOB1	TVH202	cAD1
TVA103	cOB1	TVH203	other
TVA104	cAD1	TVH204	other
TVA105	other	TVH205	cAD1
TVA111	cOB1	TVH206	other
TVA112	cAD1	TVH207	other
TVA118	cAD1	TVH208	other
TVA119	other	TVH209	cAD1
TVA124	cAD1	TVH211	cOB1
TVA125	cAD1	TVH213	cOB1
TVA126	cAD1	TVH216	cAD1
TVA152	cAD1	TVH217	cAD1
TVA153	cAD1	TVH219	cOB1
TVA158	cAD1	TVH220	cAD1
TVA160	cOB1	TVH221	cOB1
TVA162	cAD1	TVH222	other
TVA163	cAD1	TVH224	cAD1
TVA167	cAD1	TVH225	cAD1
TVA169	other	TVH226	cAD1
		TVH227	cAD1
		TVH228	cOB1

**Vancomycin resistant pheromone-responsive (cOB1) conjugative plasmids pTW11 isolated from Taiwan VRE (*E. faecalis*)**



**Analysis of the VRE isolated from human and animals in Taiwan**

- Multiple drug resistant (VCM<sup>r</sup>, EM<sup>r</sup>, BC<sup>r</sup>) pheromone-responsive conjugative plasmids were disseminated in VanA-type (Thailand-type) VRE (*E. faecalis*) strains from human and animals in Taiwan.

## Conclusion

The correlation between use of the antibiotic agents and spread (increase) of the drug resistant bacteria has been suggested. In case of VRE, the relation was very clear and VRE increased where the glycopeptide have been used. Beside by the selective pressure of the drug, the VCM<sup>r</sup> genes were rapidly disseminated among enterococci by the genetic exchange systems such as conjugative plasmids encoding multiple drug resistances.

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