

**Antibiotic resistance, biofilm formation and virulence factors activities among enterococci clinical isolates collected from two hospitals in different parts of Thailand**

Presented by

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December 13<sup>th</sup>, 2018

# Enterococci

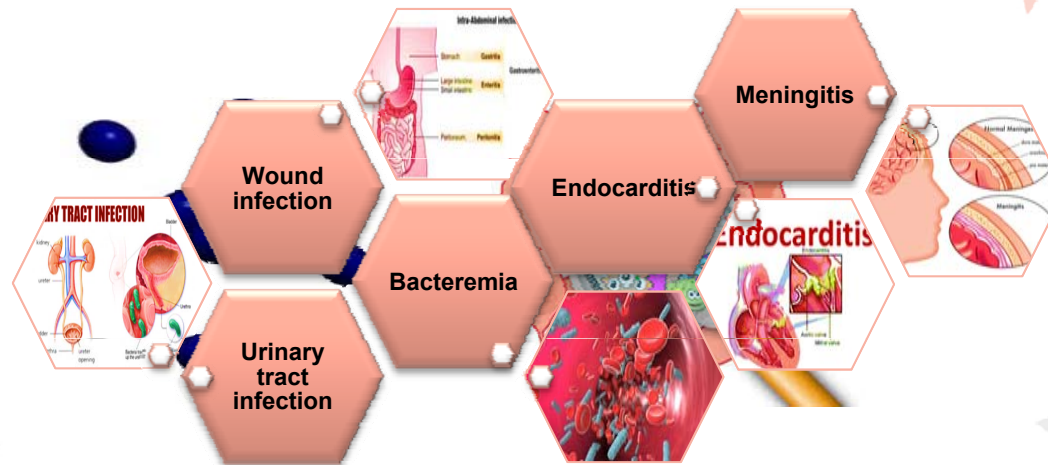


Opportunistic pathogens



*E. faecalis* and *E. faecium*

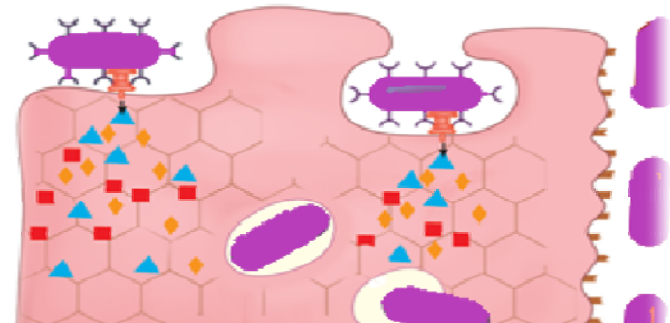
- *E. casseliflavus*, *E. gallinarum*, *E. avium*, *E. durans*,
- *E. dispar*, *E. hirae*, *E. raffinosus*, etc.



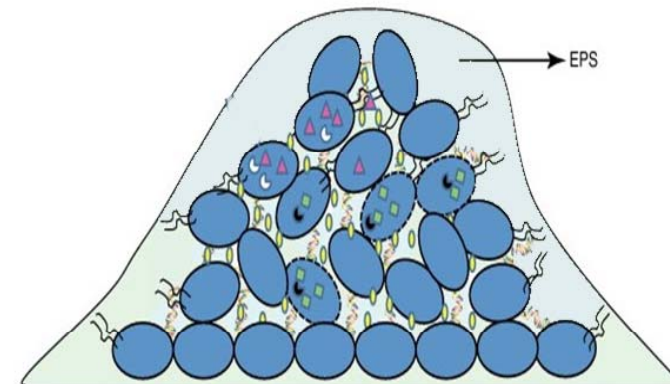
## ❑ Virulence factors of enterococci



- Secreted virulence factors
  - Cytolysin
  - Exoenzymes
    - Gelatinase
    - Caseinase
    - Lipase



- Biofilm formation



## ❑ Vancomycin-resistant enterococci (VRE)



- ❖ Co-resistance to other antibiotics leading to multidrug-resistance



WHO listed VRE *fm* as one of the high priority pathogens for the development of new antibiotics and novel strategies

[http://www.who.int/medicines/publications/WHO-PPL-Short\\_Summary\\_25Feb-ET\\_NM\\_WHO.pdf](http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25Feb-ET_NM_WHO.pdf)

[https://www.cdc.gov/drugresistance/biggest\\_threats.html](https://www.cdc.gov/drugresistance/biggest_threats.html)

December 13<sup>th</sup>, 2018

## ❑ Treatment options for VRE



Chloramphenicol

- Clinical failures, ↑ toxicity



Daptomycin

- Clinical trial, Phase III



Linezolid



Tigecycline



Quinupristin/Dalfopristin - *E. faecium*, ~~*E. faecalis*~~



Lipoglycopeptides (Telavancin, Dalbavancin, Oritavancin)



# Objectives



1

Evaluate the prevalence of antibiotic resistance and virulence factors activity of clinical enterococci isolates from two hospitals in different parts of Thailand

2

Analyze the correlation of antibiotic resistance patterns, biofilm formation and virulence factors productions

3

Characterize vancomycin-resistant enterococci from clinical isolates

# □ Sample collection



Source	Total of isolates (n)		% of isolates									
	SS	HDY	<i>E. faecalis</i>		<i>E. faecium</i>		<i>E. casseliflavus</i>		<i>E. gallinarum</i>		<i>Enterococcus spp.</i>	
			SS	HDY	SS	HDY	SS	HDY	SS	HDY	SS	HDY
Urine	406	187	65.7	73.8	31	26.2	-	-	-	-	0.2	-
Pus	77	54	83.1	88.9	13	7.4	-	-	-	-	3.9	3.7
Blood	2015 - 2017		65.5	92.9	20.7	7.1	3.5	-	3.5	-	6.9	-
Fluid	-	12	-	75	-	25	-	-	-	-	-	-
Sputum	-	5	-	80	-	20	-	-	-	-	-	-
<b>Total</b>	<b>512</b>	<b>272</b>	<b>70.7</b>	<b>77.9</b>	<b>27.7</b>	<b>21.3</b>	<b>0.2</b>	<b>-</b>	<b>0.2</b>	<b>-</b>	<b>1.2</b>	<b>0.7</b>

December 12th, 2018  
 SS – Sunpasitprasong Hospital (Northeast Thailand)

HDY – Hat Yai Hospital (Southern Thailand) 7

Antimicrobial agent	Disc content (µg)
Vancomycin	30
Teicoplanin	30
Ampicillin	10
Penicillin	10iU
Gentamicin (High-level)	120
Streptomycin (High-level)	300
Chloramphenicol	30
Ciprofloxacin	5
Erythromycin	15
Linezolid	Vancomycin-30
Tetracycline	30

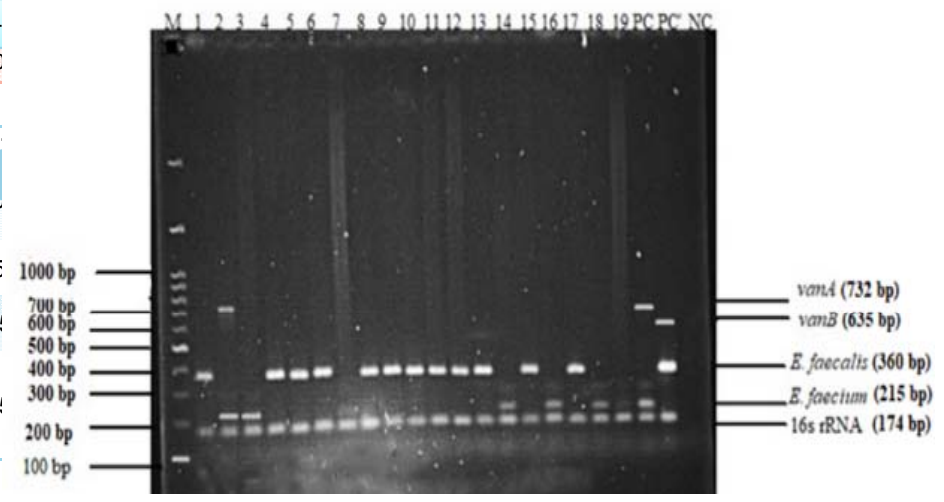
Amplify with thermal cycler  
**Primers for**  
*Enterococcus* spp.

1.5% agarose gel,  
 70V, 120 minutes  
 Vancomycin-  
 (TE)

Antimicrobial agent	Disc content (µg)
Nitrofurantoin	30
Rifampin	5
Tigecycline	1
Quinupristin/dalfopristin	1

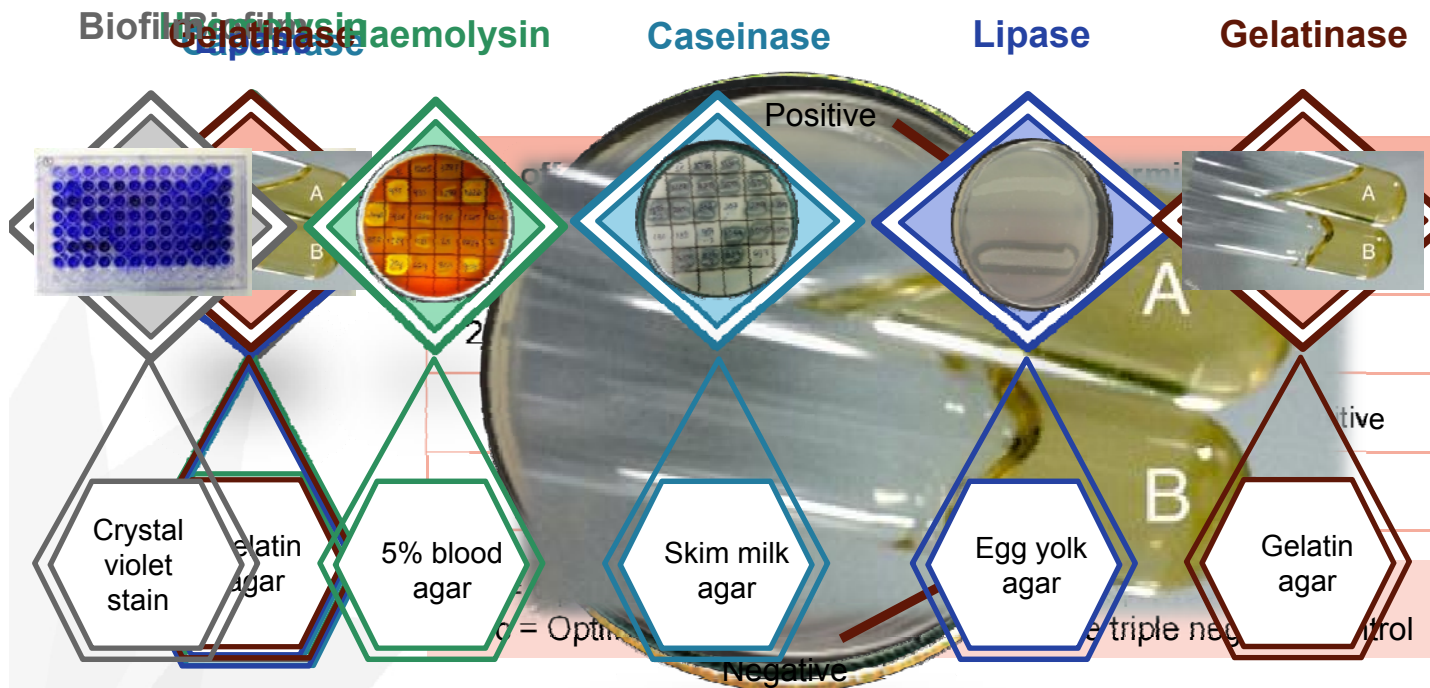
Stain with 0.5µg/ml EtBr,  
 Visualize under UV light

Name of primer	Sequence (5' - 3')	Genes
<i>E. faecalis</i> - F	ACT TAT GTG ACT AAC TTA ACC	<i>sodA</i>
<i>E. faecalis</i> - R	TAA TGG TGA ATC TTG GTT TGG	
<i>E. faecium</i> - F	GAA AAA ACA ATA GAA GAA TTA T	<i>sodA</i>
<i>E. faecium</i> - R	TGC TTT TTT GAA TTC TTC TTT A	
<i>E. casseliflavus</i> - F	TCC TGA ATT AGG TGA AAA AAC	<i>sodA</i>
<i>E. casseliflavus</i> - R	GCT AGT TTA CCG TCT TTA ACG	
<i>E. gallinarum</i> - F	TTA CTT GCT GAT TTT GAT TCG	<i>sodA</i>
<i>E. gallinarum</i> - R	TGA ATT CTT CTT TGA AAT CAG	
<i>vanA</i> - F	GGG AAA ACG ACA TTG C	<i>vanA</i>





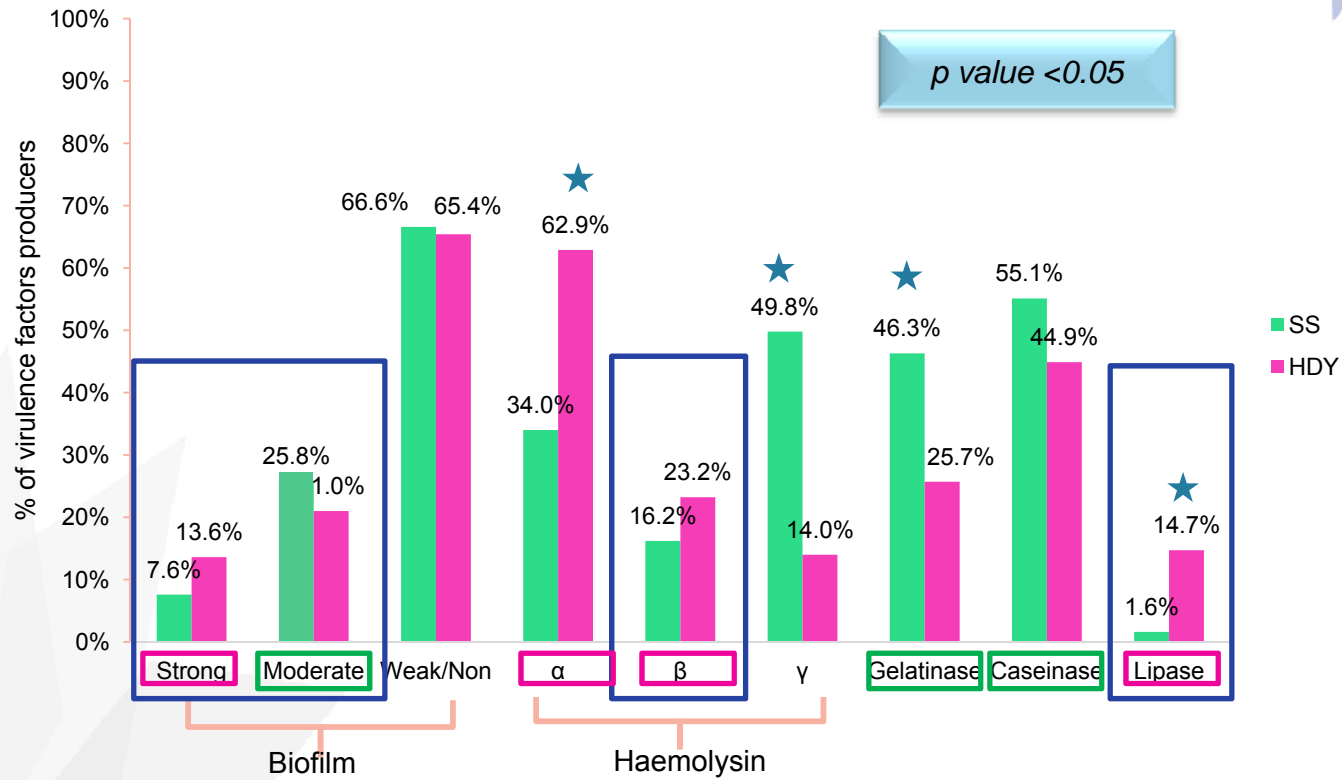
# ❑ Virulence factor activity testing



❖ Positive control – *S. aureus* ATCC 35556

❖ Positive control – *S. aureus* ATCC 25923

# Virulence factor activity testing



SS – Sunpasitthiprasong Hospital (Northeast Thailand)      HDY – Hat Yai Hospital (Southern Thailand)

## Correlation between antibiotic resistance and virulence factors

	Total isolates		Biofilm						Haemolysin						Gelatinase		Caseinase		Lipase	
			Strong		Moderate		Weak/ Non-biofilm		α		β		γ							
	SS	HDY	SS	HDY	SS	HDY	SS	HDY	SS	HDY	SS	HDY	SS	HDY	SS	HDY	SS	HDY	SS	HDY
MDR	79.9	80.5	7.2	15.9	27.6	22.4	64.2	61.7	37.3	67.7	14.3	22.4	48.3	10	45	27.4	54.5	45.8	1.8	27.4
HLAR	44.9	40.8	14.3	18	37.8	20.7	55.7	61.3	50.7	63.1	16.5	25.2	32.6	11.7	60.9	40.5	75.7	62.2	1.3	5.4
VRE	3.9	3.7	-	10	5	30	25	60	15	80	-	10	85	10	10	-	40	20	-	20

MDR – multidrug resistance

HLAR – high-level aminoglycoside resistance

VRE – vancomycin-resistant enterococci

SS – Sunpasitthiprasong Hospital (Northeast Thailand)

HDY – Hat Yai Hospital (Southern Thailand)

Resistance No.	Antibiotic resistance profiling											Source										
												Urine		Pus		Blood		Fluid	Sputum	Total		
												SS	HDY	SS	HDY	SS	HDY	HDY	HDY	SS+HDY		
10	TE	E	CIP	C	TEC	VAN	S	CN	P	AMP		2							2			
9	TE	E	CIP	TEC	VAN	S	CN	P	AMP			1	4						5			
8	TE	E	CIP	C	S	CN	P	AMP				22	5	1	1	1			30			
8	TE	E	CIP	TEC	VAN	CN	P	AMP				10		1					11			
7	TE	E	CIP	C	S	CN	P					94	12	5	1	5	1	2	1	121		
7	TE	E	CIP	S	CN	P	AMP					7	10	1	1		1	2		22		
6	TE	E	CIP	C	S	CN						30	19	3	7		1	1		61		
6	TE	E	CIP	C	CN	P						2	1				1	1		5		
6	TE	E	CIP	C	P	AMP						2	2		1					5		
6	TE	E	CIP	S	CN	P						18	6		1					25		
6	TE	E	CIP	S	P	AMP						1	4	1	2		1			9		
6	TE	E	CIP	CN	P	AMP						26	9	1	2	1	1			40		
6	E	CIP	C	S	CN	P						5								5		
6	E	CIP	S	CN	P	AMP						3	4	2				1		10		
5	TE	E	CIP	S	CN							7	7		1				2	17		
5	TE	E	CIP	P	AMP							25	3	2	1	2				33		
5	TE	E	CIP	C	CN								5	1	1		2			9		
5	TE	E	CIP	C	S								3	1		2				6		
5	TE	E	C	S	CN							6	3	4	1	2				16		
5	E	CIP	S	P	AMP							7	5							12		
5	E	CIP	C	S	CN							1	3							4		
5	E	CIP	CN	P	AMP							14	1	2	1					18		
4	TE	E	CIP	C			TE	- tetracycline				3	1								4	
4	TE	E	CIP	S			E	- erythromycin				1	4								5	
4	TE	E	CIP	CN			CIP	- ciprofloxacin				1	2		1						4	
4	TE	E	C	S			C	- chloramphenicol				5	3		1						9	
4	TE	E	C	CN			TEC	- teicoplanin				6		1		1					8	
4	TE	E	S	CN			VAN	- vancomycin				2	5	3	2						12	
4	TE	CIP	P	AMP			S	- streptomycin				4							1		4	
4	E	CIP	P	AMP			CN	- gentamicin				18	6	3		1					28	
3	TE	E	S				P	- penicillin				3	1	3	1						8	
3	TE	E	CN				AMP	- ampicillin				2		1	2	1				1		7

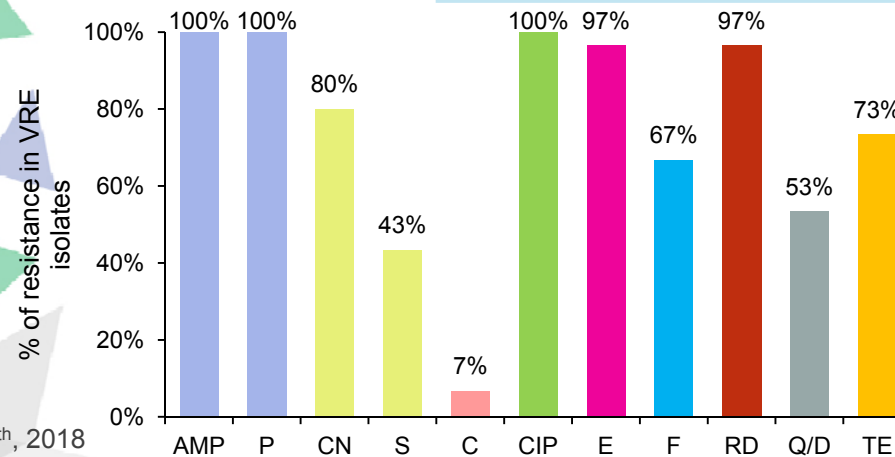
## ❑ Vancomycin-resistant enterococci

Species	Urine (n)	Pus (n)	Fluid (n)	Genotype	MIC (µg/ml)
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**Linezolid and tigecycline are 100% sensitive in VRE isolates**

<i>E. casseliflavus</i>	-	-	-		
<i>E. gallinarum</i>	-	-	-		

VAN (Resistance) -  $\geq 32$  µg/ml (CLSI,2017)



AMP	- ampicillin
P	- penicillin
CN	- gentamicin
S	- streptomycin
C	- chloramphenicol
CIP	- ciprofloxacin
E	- erythromycin
F	- nitrofurantoin
RD	- rifampin
Q/D	- quinupristin/dalfopristin
TE	- tetracycline

December 13<sup>th</sup>, 2018



## □ Conclusions



*E. faecium* were more resistant to antibiotics than *E. faecalis*

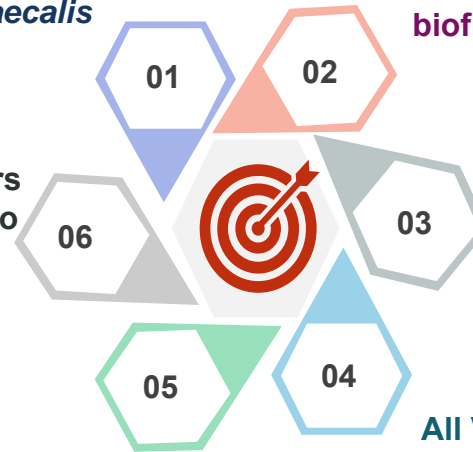
*E. faecalis* were more produced biofilm and virulence factors than *E. faecium*

Antibiotic resistant rates were similar although virulence factors productions were different in two hospitals in Thailand

vanA genotype- vanB phenotype was reported in Thailand

> 70% of clinical enterococci isolates were MDR

All VRE were MDR but linezolid and tigecycline were 100% sensitive in VRE



## □ Acknowledgements



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Ms. Kanitta Muangngam



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TEH-AC scholarship  
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Department of Microbiology, Prince of Songkla University

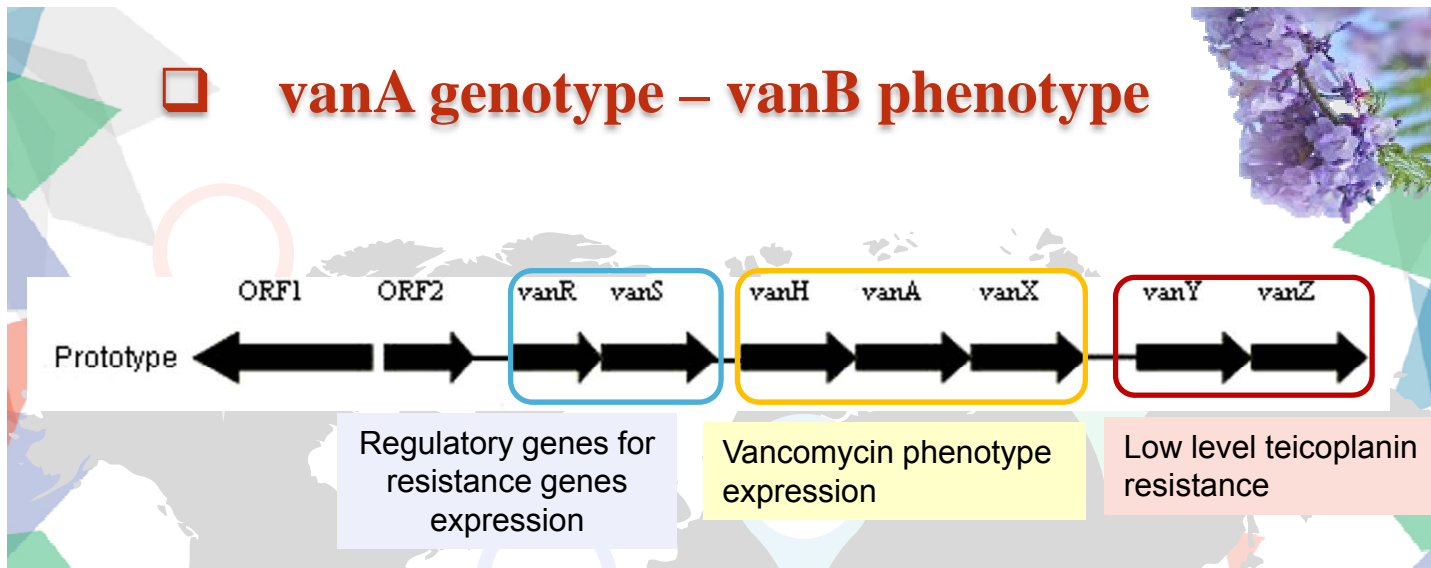


**Thank you for your attention!!**





## □ *vanA* genotype – *vanB* phenotype



- Point mutations in sensor domain of *vanS* gene
- Impairment of accessory proteins *vanY* and *vanZ* in the Tn1546 like element



## Vancomycin-Resistant Enterococci from Humans and Retail Chickens in Taiwan with Unique VanB Phenotype-*vanA* Genotype Incongruence

Tsai-Ling Lauderdale,<sup>1\*</sup> L. Clifford McDonald,<sup>2</sup> Yih-Ru Shiau,<sup>1</sup> Pei-Chen Chen,<sup>1</sup>  
Hui-Yin Wang,<sup>1</sup> Jui-Fen Lai,<sup>1</sup> and Monto Ho<sup>1</sup>

Taiwan, 2002 – 39 VRE isolated from chicken carcasses and 4 human VRE isolates


Point mutations in the *vanS* gene

## Emergence of *vanA* Genotype Vancomycin-Resistant Enterococci with Low or Moderate Levels of Teicoplanin Resistance in Korea

Joong-Sik Eom,<sup>1</sup> In-Sook Hwang,<sup>2</sup> Byung-Yoen Hwang,<sup>2</sup> Jae-Gab Lee,<sup>2</sup> Yeon-Joo Lee,<sup>2</sup>  
Hee-Jin Cheong,<sup>2</sup> Yong-Ho Park,<sup>3</sup> Seung-Chul Park,<sup>2</sup> and Woo-Joo Kim<sup>2\*</sup>

Korea, 2004 – 9 *vanA* genotype show vanB phenotype from urine, pus, blood isolates

Point mutations in the *vanS* gene



A new Tn1546 type of VanB phenotype–*vanA* genotype  
vancomycin-resistant *Enterococcus faecium* isolates in mainland China

Li Gu<sup>a,1</sup>, Bin Cao<sup>b,1</sup>, Yingmei Liu<sup>b</sup>, Ping Guo<sup>b</sup>, Shufan Song<sup>b</sup>, Ran Li<sup>b</sup>,  
Huaping Dai<sup>a</sup>, Chen Wang<sup>a,\*</sup>

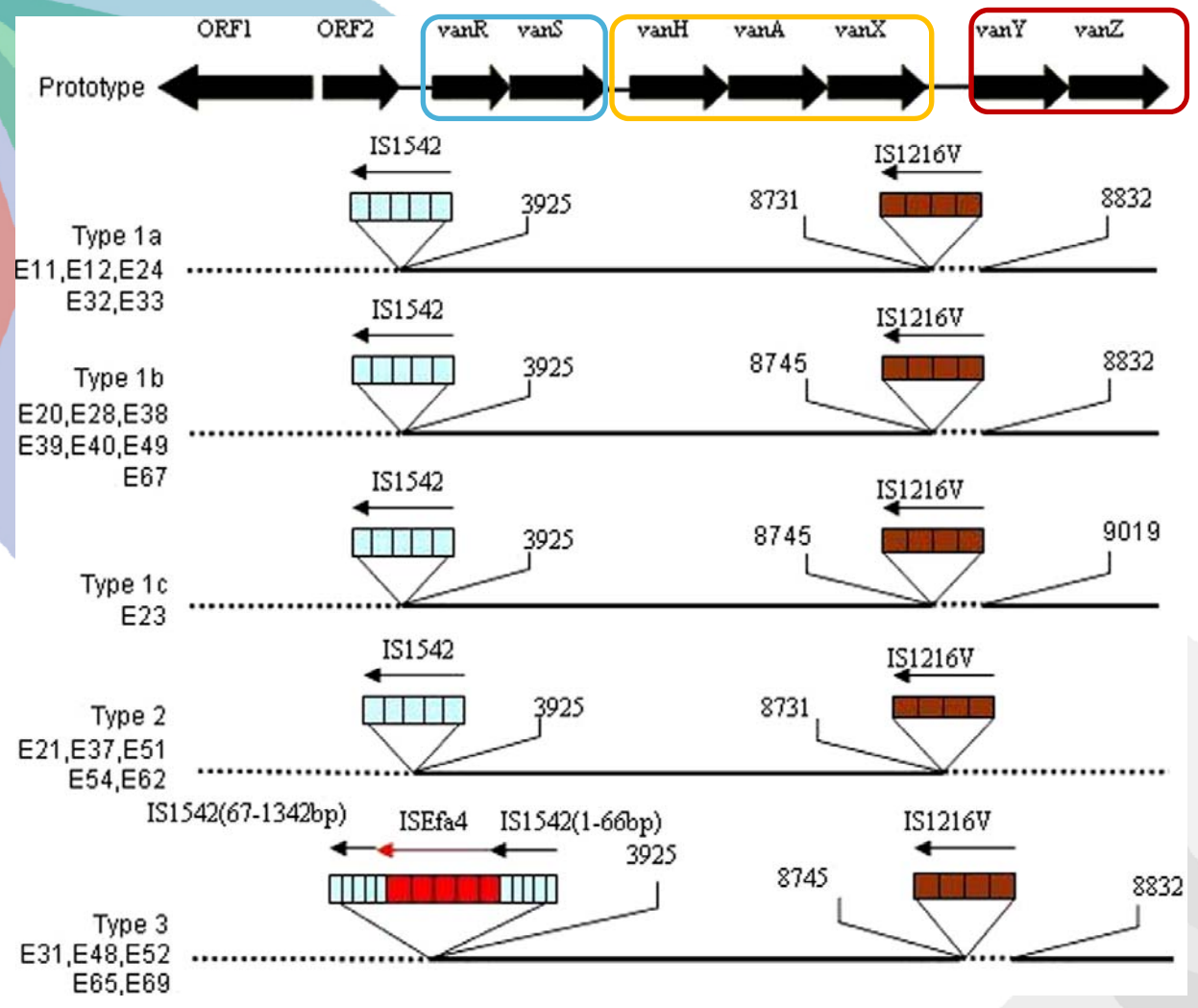
China, 2009 - 12 out of 23 *vanA* genotype show vanB phenotype

The deletion of *vanY* and *vanZ* genes or *ISEfa4* insertion in *orf2-vanR* intergenic region

**Emergence of VanB phenotype-*vanA* genotype  
*Enterococcus faecium* clinical isolate in Bulgaria**

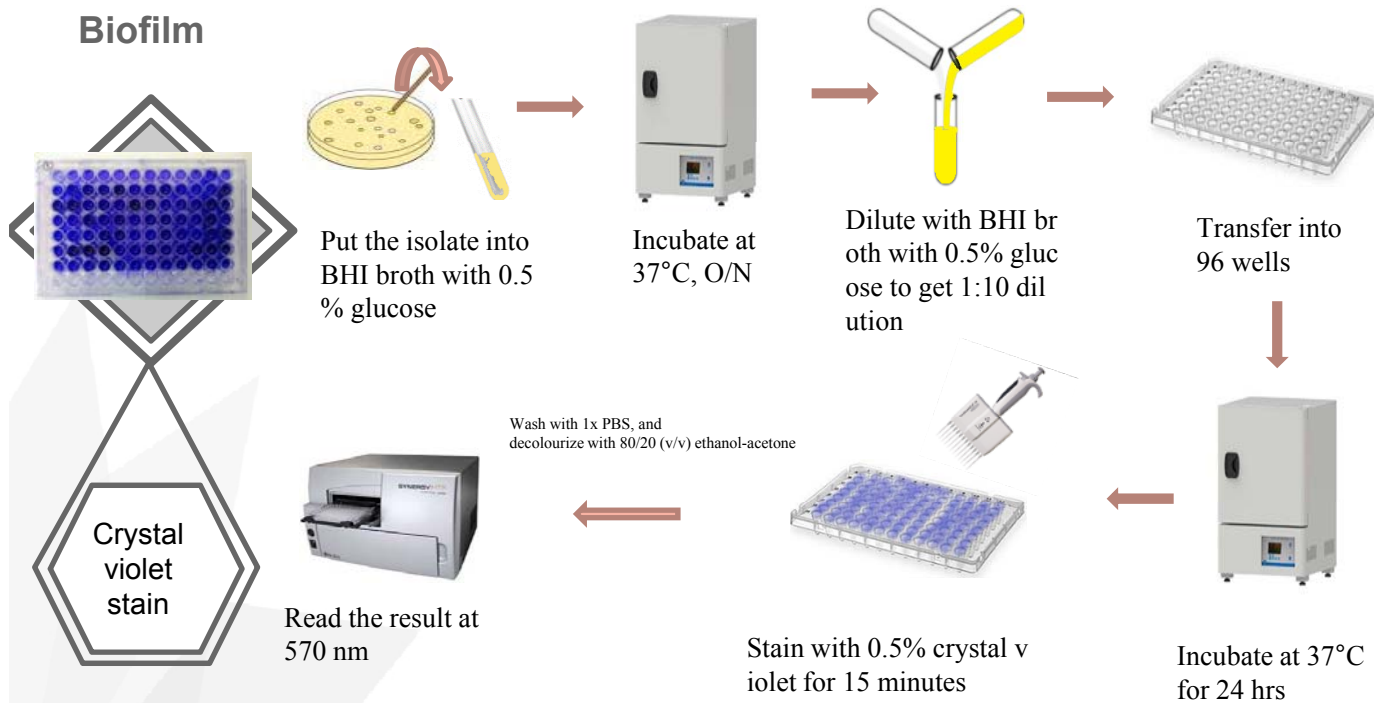
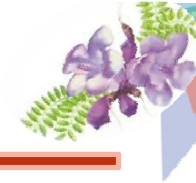
Bulgaria, 2014 – 1 *vanA* genotype show vanB phenotype from blood isolates

Point mutations in the *vanS* gene



Resistance	High		Variable	Moderate	Low				Low
	VanA	VanM	VanB	VanD	VanE	VanG	VanL	VanN	VanC
<b>Vancomycin MIC(mg/L)</b>	64 - 1,000	>256	4 - 1,000	64 - 128	8-32	≤16	8	16	2-32
<b>Teicoplanin MIC (mg/L)</b>	16 - 512	96	0.5 - 1	4- 64	0.5	0.5	≤0.5	≤0.5	0.5 - 1
<b>Modification</b>	← D-Ala-D-Lac →				← D-Ala-D-Ser →				
<b>Location</b>	Plasmid/ Chromosome	Plasmid/ Chromosome	Plasmid/ Chromosome	Plasmid/ Chromosome	Chromosome	Chromosome	Chromosome	Plasmid	Chromosome
<b>Transferrable</b>	Yes	Yes	Yes	No	No	No	No	Yes	No
<b>Expression</b>	Inducible	Inducible	Inducible	Constitutive or inducible	Inducible	Inducible	Inducible	Constitutive	Constitutive or inducible
<b>Species</b>	<i>E. faecalis</i> , <i>E. faecium</i> , <i>E. gallinarum</i> , <i>E. casseliflavus</i> , <i>E. durans</i> ,	<i>E. faecium</i>	<i>E. faecalis</i> , <i>E. faecium</i>	<i>E. faecalis</i> , <i>E. faecium</i>	<i>E. faecalis</i>	<i>E. faecalis</i>	<i>E. faecalis</i>	<i>E. faecium</i>	<i>E. gallinarum</i> , <i>E. casseliflavus</i> , <i>E. flavescentis</i>

## □ Biofilm assay



## Classification of biofilm-forming ability in microtitre plate assay

Cut-off value calculation	Biofilm-forming ability
$OD > 4 \times OD_c$	Strong
$2 \times OD_c < OD \leq 4 \times OD_c$	Moderate
$OD_c < OD \leq 2 \times OD_c$	Weak
$OD < OD_c$	None

OD = Optimal density of average value of the triple test

OD<sub>c</sub> = Optimal density of average value of the triple negative control

# Haemolysin test



MHA



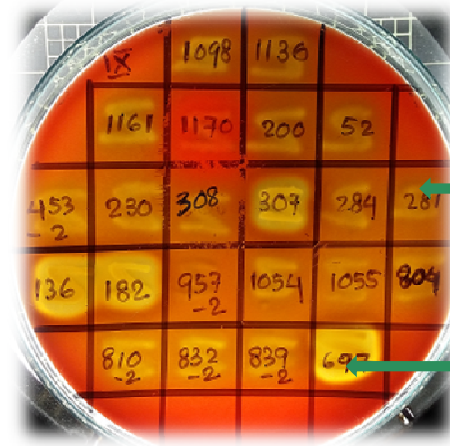
5% blood



Muller Hinton  
Agar



Incubate at 37°C  
for 24 hrs



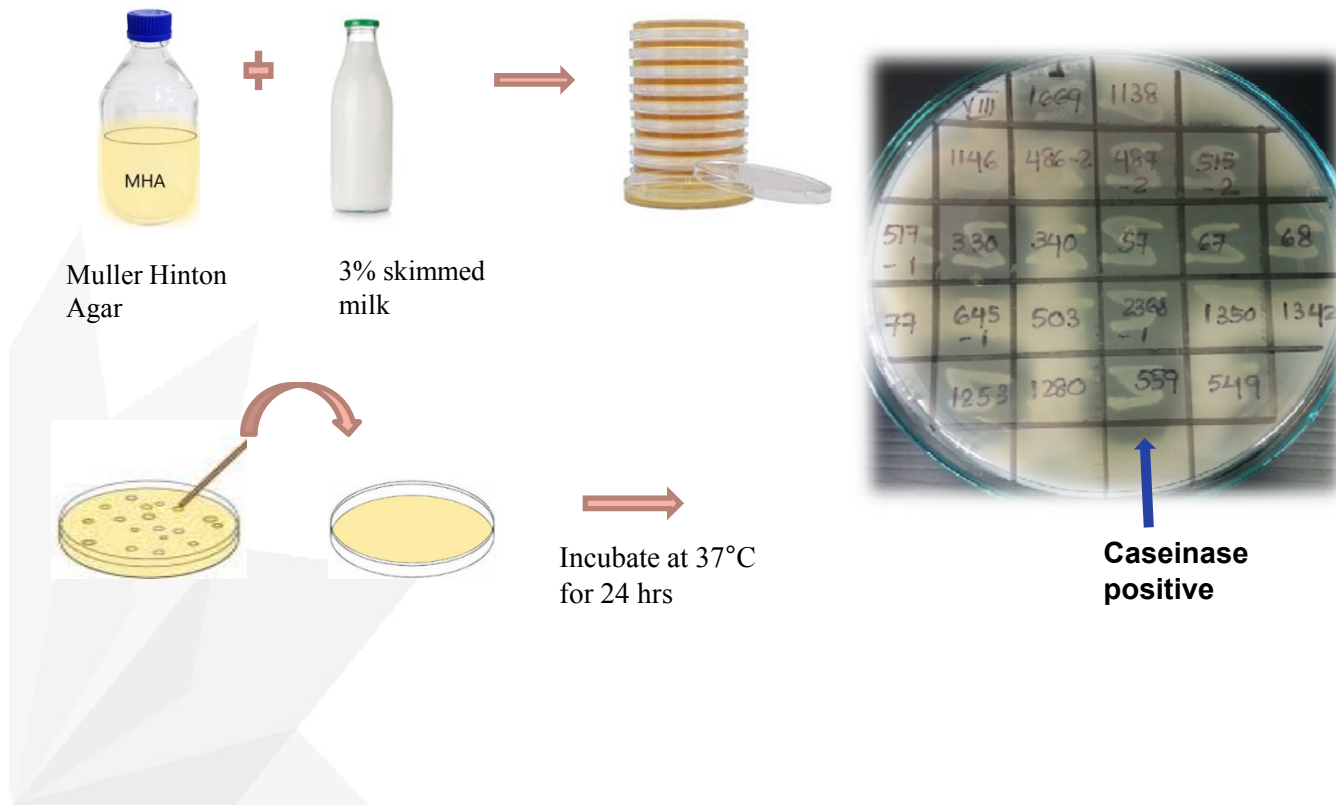
α-haemolysi

β-haemolysi

❖ Positive control – *Stap. aureus* ATCC 25923



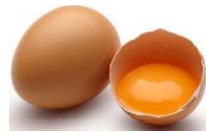
# Caseinase Test



## □ Lipase Test

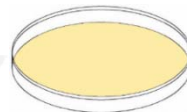


+

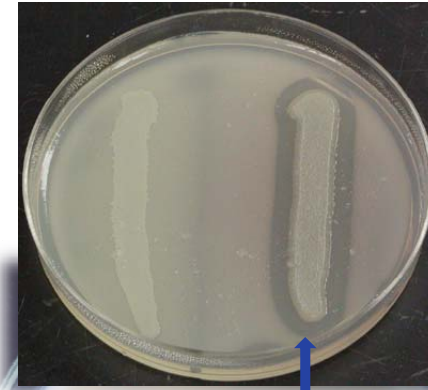


Muller Hinton  
Agar

50% egg yolk emuls  
ion



Incubate at 37°C  
for 24 – 48 hrs



**Lipase Positive**

## □ Gelatinase Test



Prepare 1 ml gelatin agar



Stab the colony into the gelatin tube

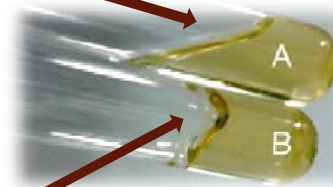


Incubate at 37°C for 24 hrs



Put into the refrigerator at least 30 mins or until NC solidify

**Positive**

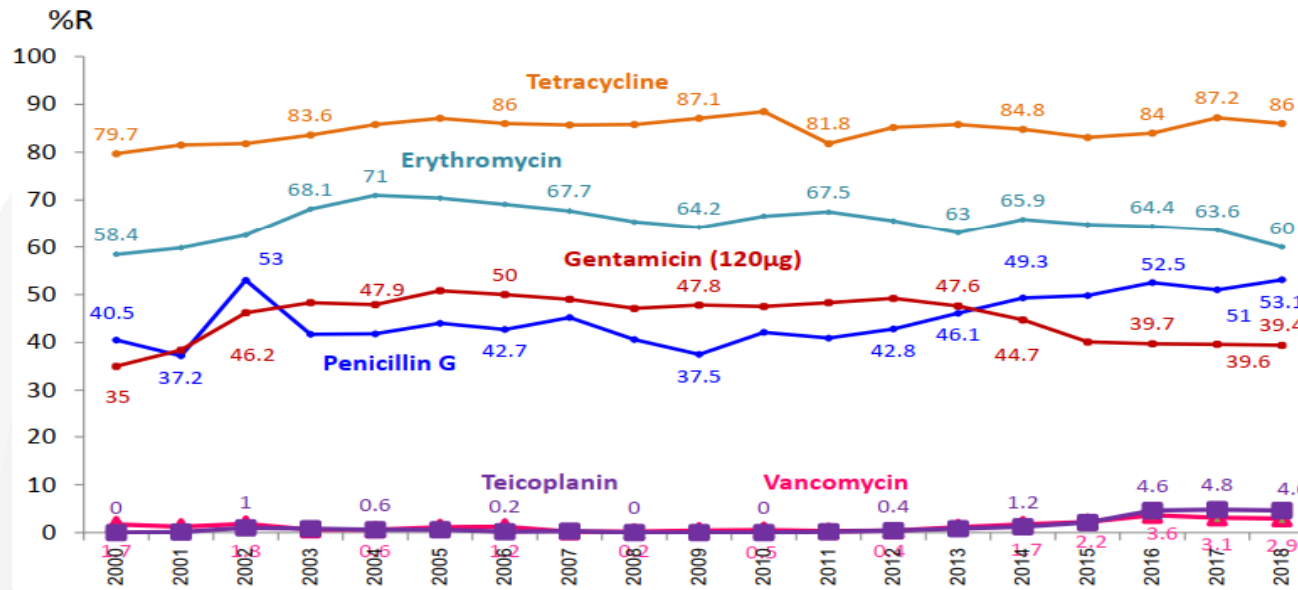


**Negative**

Examine the liquefaction of the gelatin medium

☐ Antibiotic resistance rates of *Enterococcus* spp. in Thailand

Antimicrobial Resistance rates of *Enterococcus* spp. by year  
(NARST - 69 hospitals, 6M 2018)





**Jacaranda filicifolia**  
**(Sritrang tree)**

Indigenous tree of Brazil