From Antimicrobial Susceptibility Test of Antimicrobial Resistance to The Genetic Epidemiology

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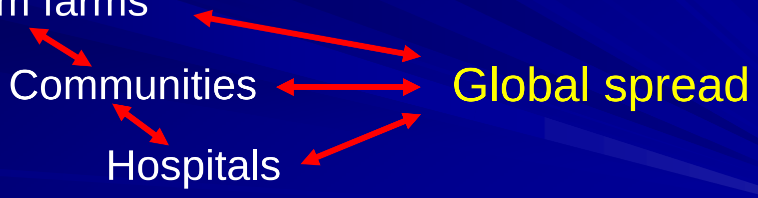
Outline

- Antimicrobial resistance and their impact
- Laboratory and technologies
- Mechanisms of resistance and detection problems
- Spread of seriously resistant bacteria in communities
- How to control and policy making

Key Messages

- Severe antibiotic resistance problems
- Resistance emerges from selection pressure
- From “Vertical” to “Horizontal” gene transfer
- Hospitals are the final destinations
- From farms



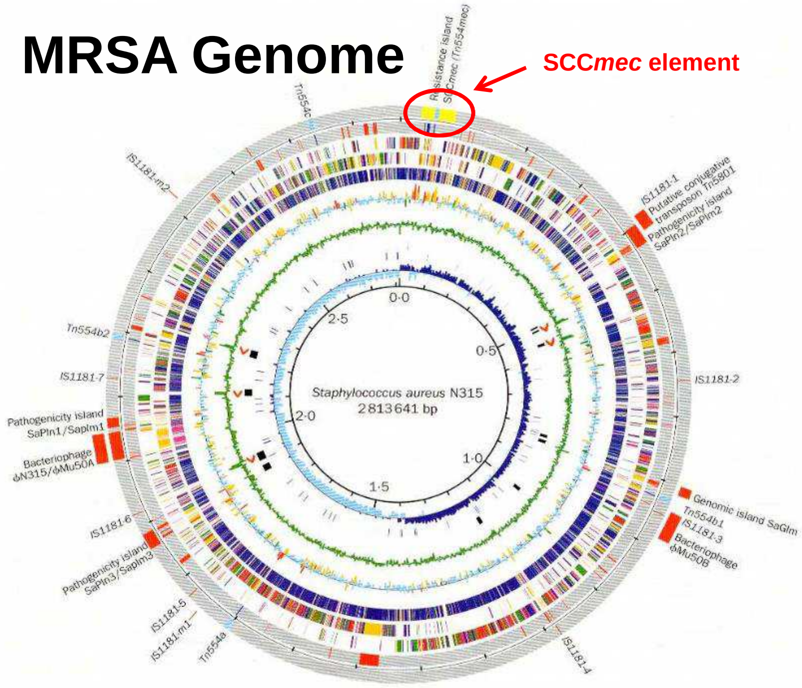
Communities

Global spread

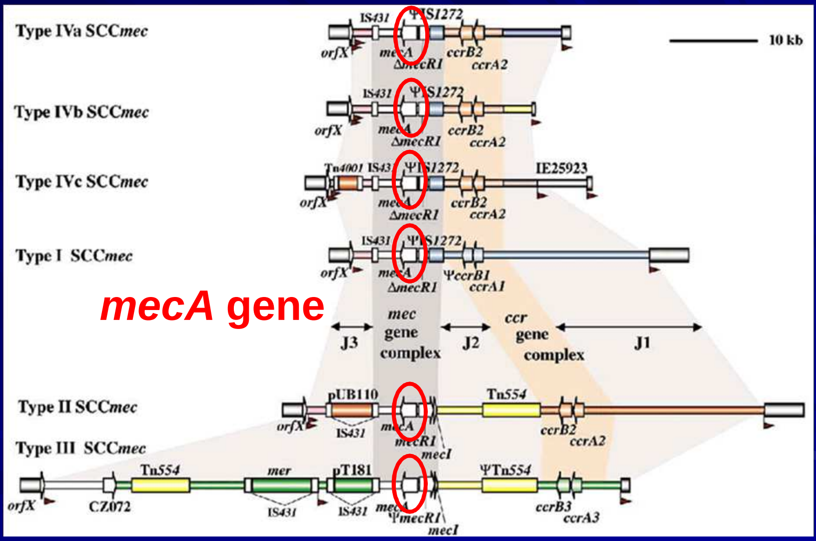
Hospitals

- Molecular epidemiology is the key

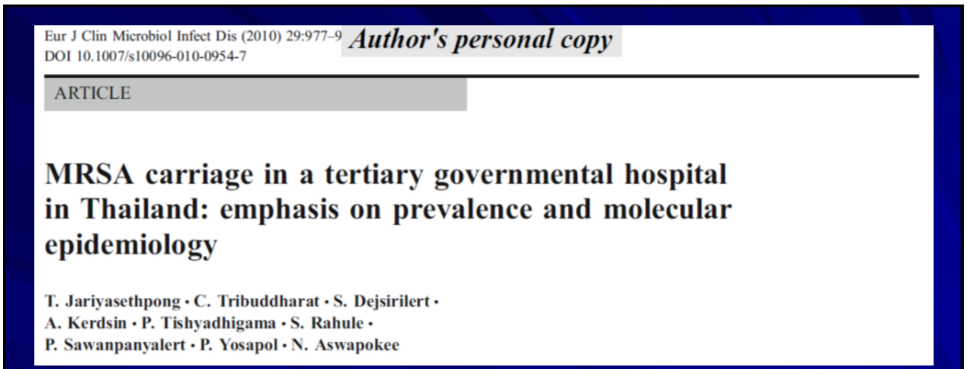
MRSA Genome



SCC*mec* element



Classification of SCC*mec* (Ito T *et al.* Insight on antibiotic resistance of *Staphylococcus aureus* form its whole genome: genomic island SCC. Drug Resist Updates 2003;6:41-52)



Eur J Clin Microbiol Infect Dis (2010) 29:977–9
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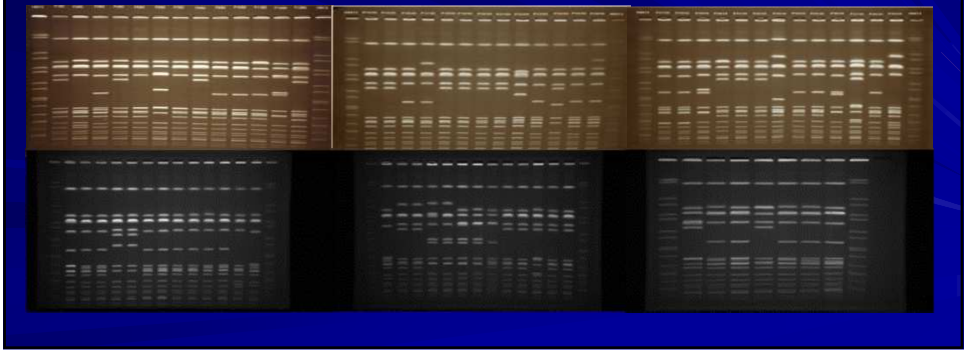
ARTICLE

MRSA carriage in a tertiary governmental hospital in Thailand: emphasis on prevalence and molecular epidemiology

T. Jariyasethpong · C. Tribuddharat · S. Dejsirilert · A. Kerdsin · P. Tishyadhigama · S. Rahule · P. Sawanpanyalert · P. Yosapol · N. Aswapokee

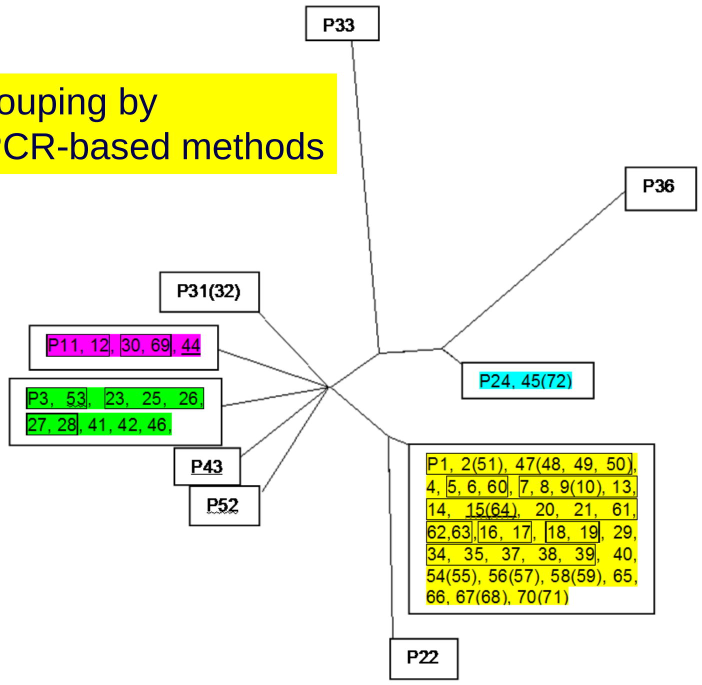


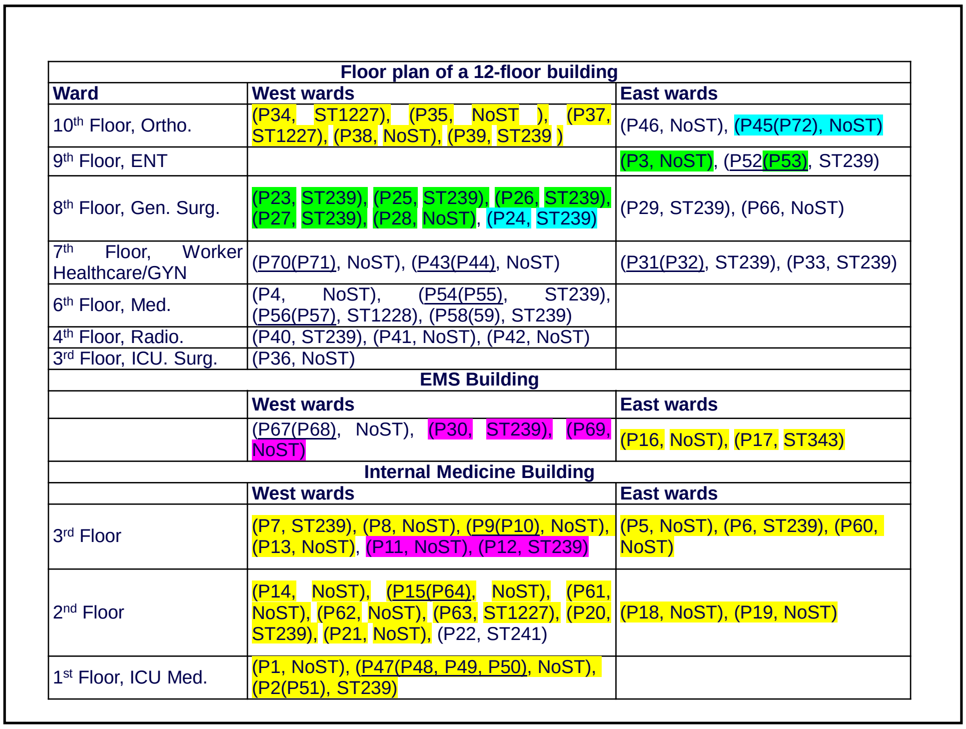
PCR-based Typing Method

Multi-locus Sequence Typing (MLST)

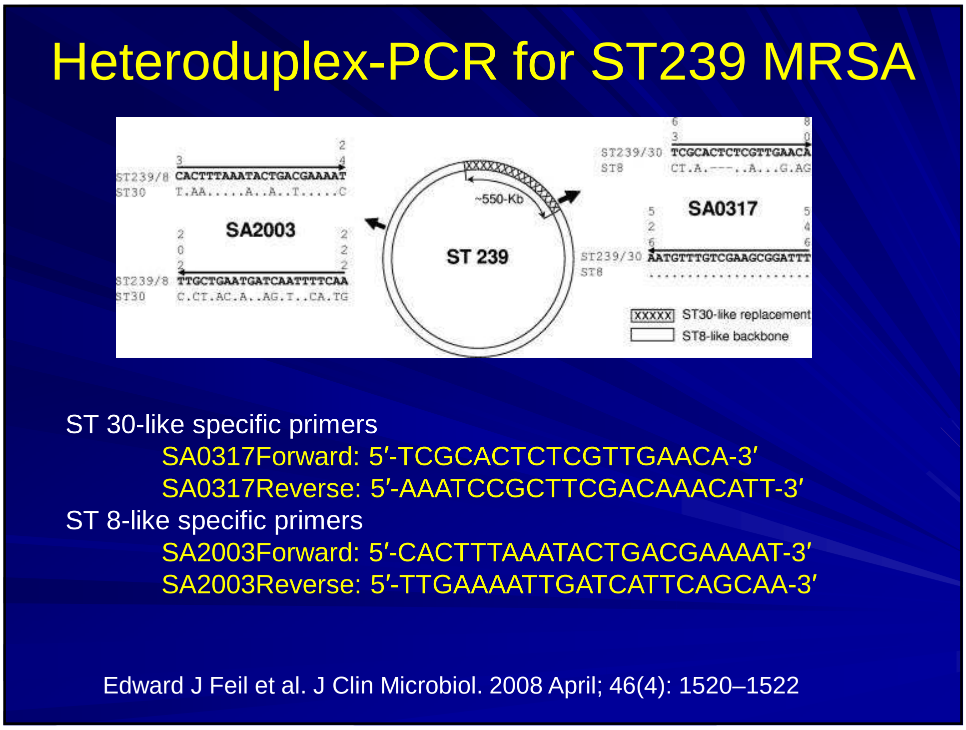
Lab no	SCC mec	Spa type	Coa type	HVR DRU	PFGE type	ST type	Allelic Profiles						
							arcc	aroe	glpf	gmk	pta	tpi	yqil
P24	III	A	B	13	C	239	2	3	1	1	4	4	3
P54	III	A	B	7	D	239	2	3	1	1	4	4	3
P51	III	A	B	7	A8	239	2	3	1	1	4	4	3
P29	III	A	B	7	J	239	2	3	1	1	4	4	3
P40	III	A	B	7	B2	239	2	3	1	1	4	4	3
P12	III	A	B	5	J	239	2	3	1	1	4	4	3
P31	III	A	B	6	A1	239	2	3	1	1	4	4	3
P26	III	A	B	15	K2	239	2	3	1	1	4	4	3
P20	III	A	B	7	A3	239	2	3	1	1	4	4	3
P23	III	A	B	15	H	239	2	3	1	1	4	4	3
P7	III	A	B	7	A2	239	2	3	1	1	4	4	3
P39	III	A	B	7	E1	239	2	3	1	1	4	4	3
P52	III	A	B	3	F	239	2	3	1	1	4	4	3
P33	III	A	A	9	P	239	2	3	1	1	4	4	3
P25	III	A	B	15	K1	239	2	3	1	1	4	4	3
P30	III	A	B	5	A5	239	2	3	1	1	4	4	3
P6	III	A	B	7	O	239	2	3	1	1	4	4	3
P27	III	A	B	15	B1	239	2	3	1	1	4	4	3
P22	III	A	D	7	G	241	2	3	1	1	4	4	30
P17	III	A	B	7	E2	343	2	85	1	1	4	4	3
P34	III	A	B	7	A7	1227	149	3	1	1	4	4	3
P37	III	A	B	7	I	1227	149	3	1	1	4	4	3
P63	III	A	B	7	A4	1227	149	3	1	1	4	4	3
P56	III	A	B	7	N	1228	2	188	1	1	4	4	3

Grouping by Combined PCR-based methods





Floor plan of a 12-floor building		
Ward	**West wards**	**East wards**
10th Floor, Ortho.	(P34, ST1227), (P35, NoST), (P37, ST1227), (P38, NoST), (P39, ST239)	(P46, NoST), (P45(P72), NoST)
9th Floor, ENT		(P3, NoST), (P52(P53), ST239)
8th Floor, Gen. Surg.	(P23, ST239), (P25, ST239), (P26, ST239), (P27, ST239), (P28, NoST), (P24, ST239)	(P29, ST239), (P66, NoST)
7th Floor, Worker Healthcare/GYN	(P70(P71), NoST), (P43(P44), NoST)	(P31(P32), ST239), (P33, ST239)
6th Floor, Med.	(P4, NoST), (P54(P55), ST239), (P56(P57), ST1228), (P58(59), ST239)	
4th Floor, Radio.	(P40, ST239), (P41, NoST), (P42, NoST)	
3rd Floor, ICU. Surg.	(P36, NoST)	
EMS Building		
	West wards	**East wards**
	(P67(P68), NoST), (P30, ST239), (P69, NoST)	(P16, NoST), (P17, ST343)
Internal Medicine Building		
	West wards	**East wards**
3rd Floor	(P7, ST239), (P8, NoST), (P9(P10), NoST), (P13, NoST), (P11, NoST), (P12, ST239)	(P5, NoST), (P6, ST239), (P60, NoST)
2nd Floor	(P14, NoST), (P15(P64), NoST), (P61, NoST), (P62, NoST), (P63, ST1227), (P20, ST239), (P21, NoST), (P22, ST241)	(P18, NoST), (P19, NoST)
1st Floor, ICU Med.	(P1, NoST), (P47(P48, P49, P50), NoST), (P2(P51), ST239)	

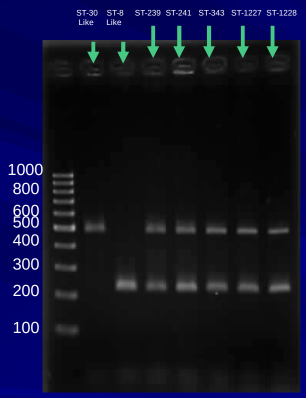


Heteroduplex-PCR for ST239 MRSA

ST 30-like specific primers
    SA0317Forward: 5′-TCGCACTCTCGTTGAACA-3′
    SA0317Reverse: 5′-AAATCCGCTTCGACAAACATT-3′
ST 8-like specific primers
    SA2003Forward: 5′-CACTTTAAATACTGACGAAAAT-3′
    SA2003Reverse: 5′-TTGAAAATTGATCATTCAGCAA-3′

Edward J Feil et al. J Clin Microbiol. 2008 April; 46(4): 1520–1522

Heteroduplex PCR for ST239 Lineage

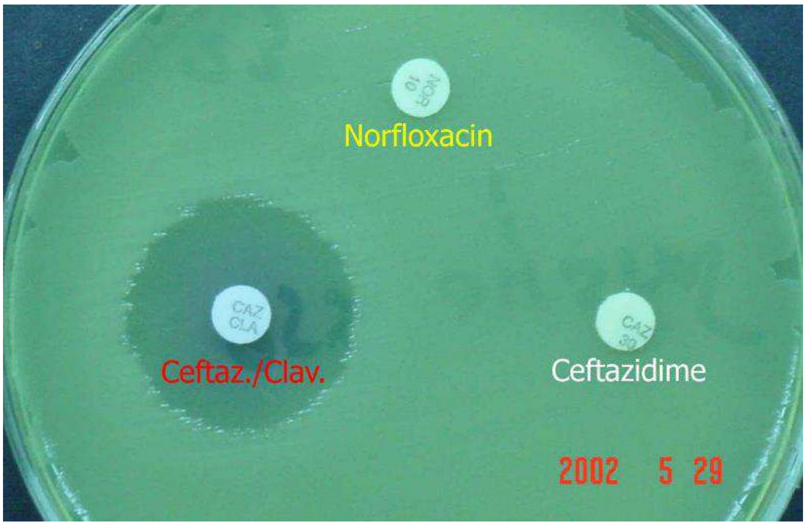


ST-30 Like = *S. aureus* Cowan I
ST-8 Like = MRSA SCC*mec* IV
ST239 = P24
ST241 = P22
ST343 = P17
ST1227 = P37
ST1228 = P56
ST8 F: 5′-CACTTTAAATACTGACGAAAAT-3′
R: 5′-TTGAAAATTGATCATTC AGCAA-3′
ST30 F: 5′-TCGCACTCTCGT TGAACA-3′
R: 5′-AAATCCGCTTCGACAAACATT-3′

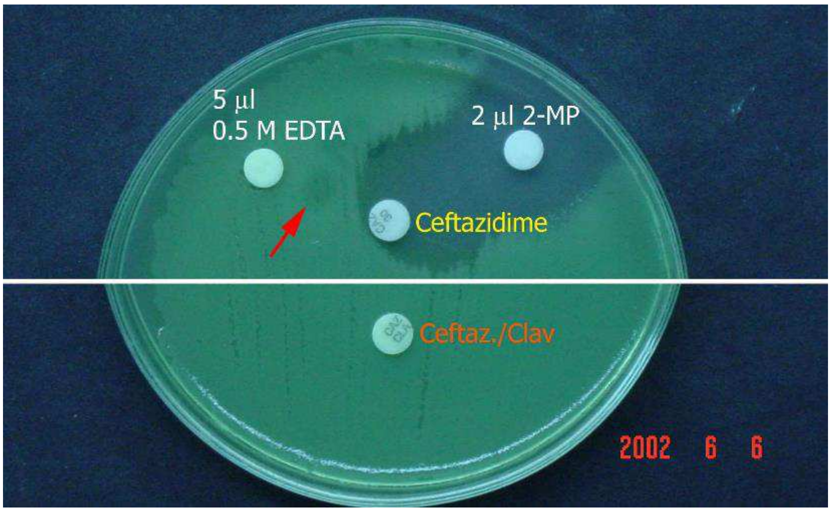
The identification of the ST8 and ST30 recombinant chromosome.
PCR of 484 bp = ST30-like chromosomal segment and 220 bp = ST8-like segment.

T. Jariyasethpong, C. Tribuddharat, S. Dejsirilert, et al: Eur J Clin Microbiol Infect Dis (2010) 29:977–9

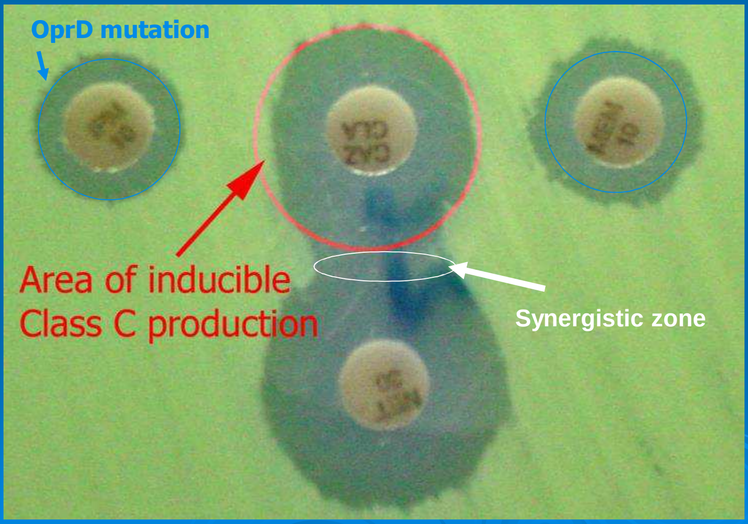
Class A Producer



Class B Producer

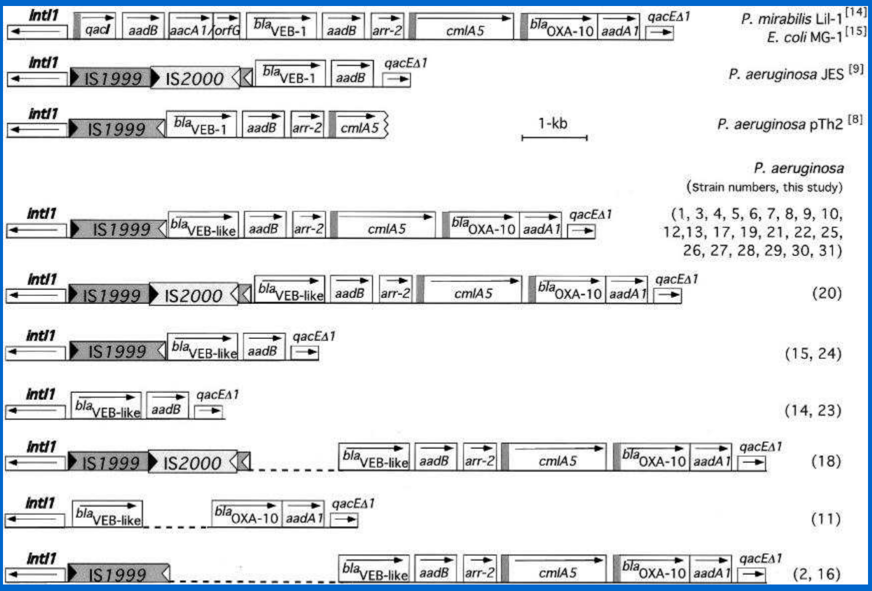


Class A and C β-lactamase Producer and Some Extra Findings



Green colony of ***Pseudomonas aeruginosa*** **No.3**

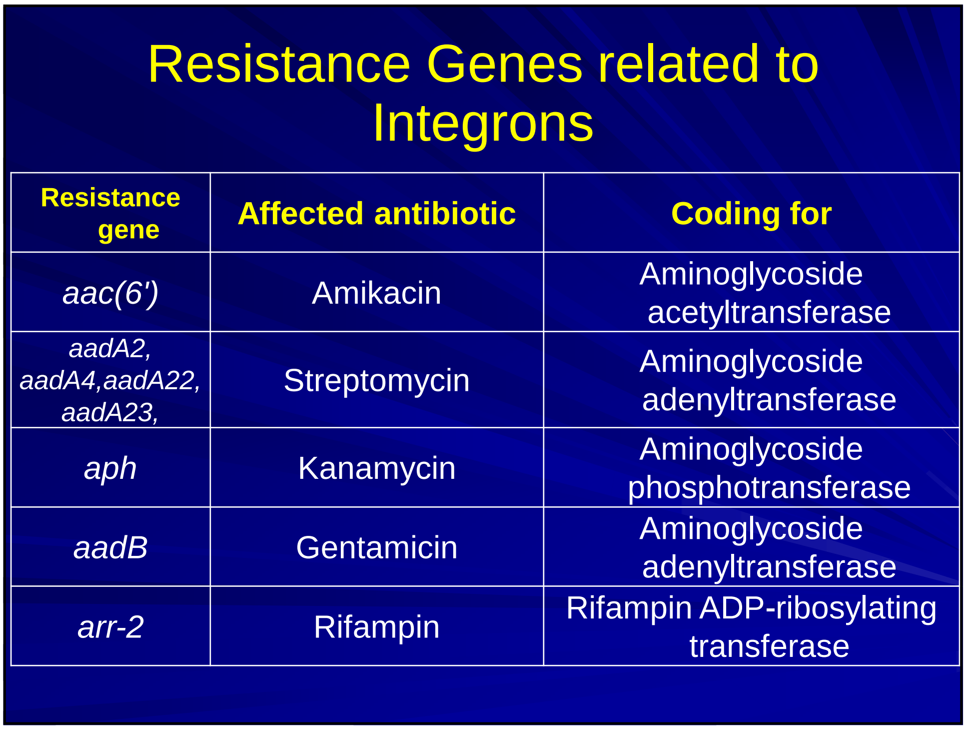
Genetic Variations of Integrons



Clin. Infect. Dis. 2002;34:603-611, Girlich *et al.*

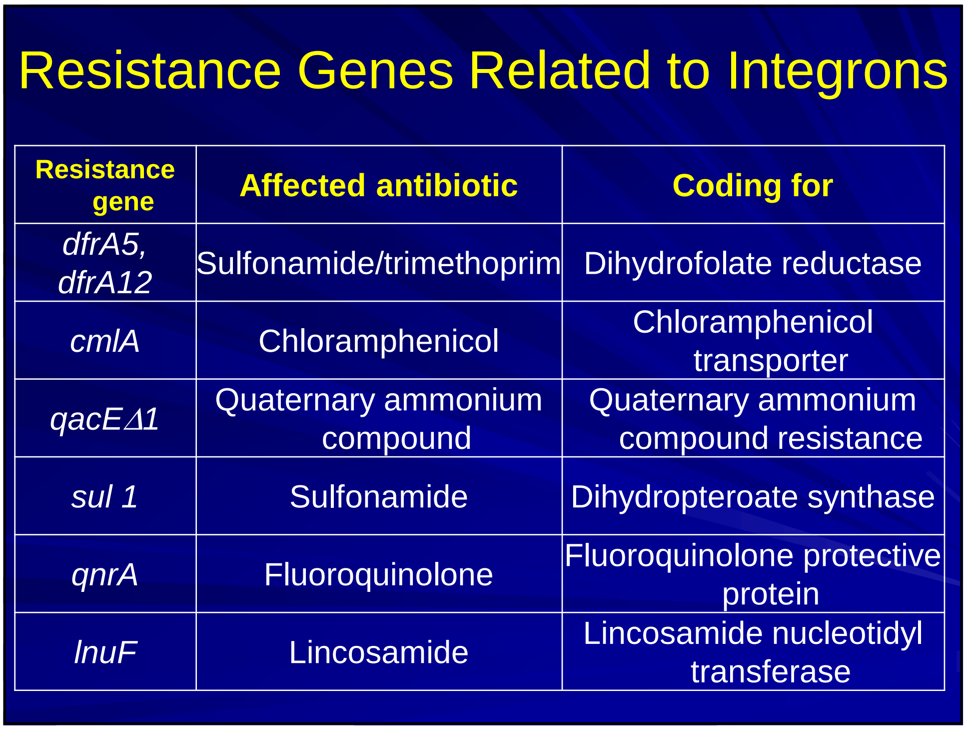
Identified Resistance Genes related to Integrons Thai clinical isolates

Resistance gene	**Affected antibiotic**	**Coding for**
$bla_{\text{IMP-14}}$	Carbapenem	Metallo-β-lactamase
$bla_{\text{IMP-15}}$	Carbapenem	Metallo-β-lactamase
$bla_{\text{IMP-32}}$	Carbapenem	Metallo-β-lactamase
$bla_{\text{VEB-1}}$	Cephalosporin	Class A β-lactamase
$bla_{\text{PSE-1}}$	Carbenicillin	Class A β-lactamase
$bla_{\text{OXA-10}}$	Oxacillin	Class D β-lactamase



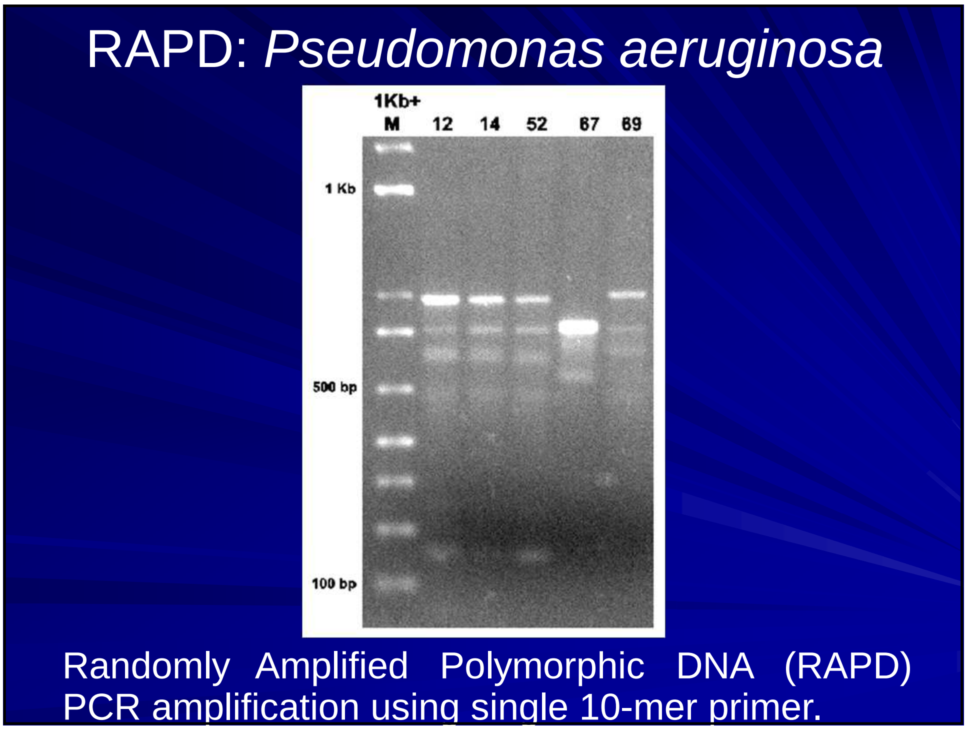
Resistance Genes related to Integrons

Resistance gene	**Affected antibiotic**	**Coding for**
aac(6')	Amikacin	Aminoglycoside acetyltransferase
aadA2, aadA4,aadA22, aadA23,	Streptomycin	Aminoglycoside adenyltransferase
aph	Kanamycin	Aminoglycoside phosphotransferase
aadB	Gentamicin	Aminoglycoside adenyltransferase
arr-2	Rifampin	Rifampin ADP-ribosylating transferase



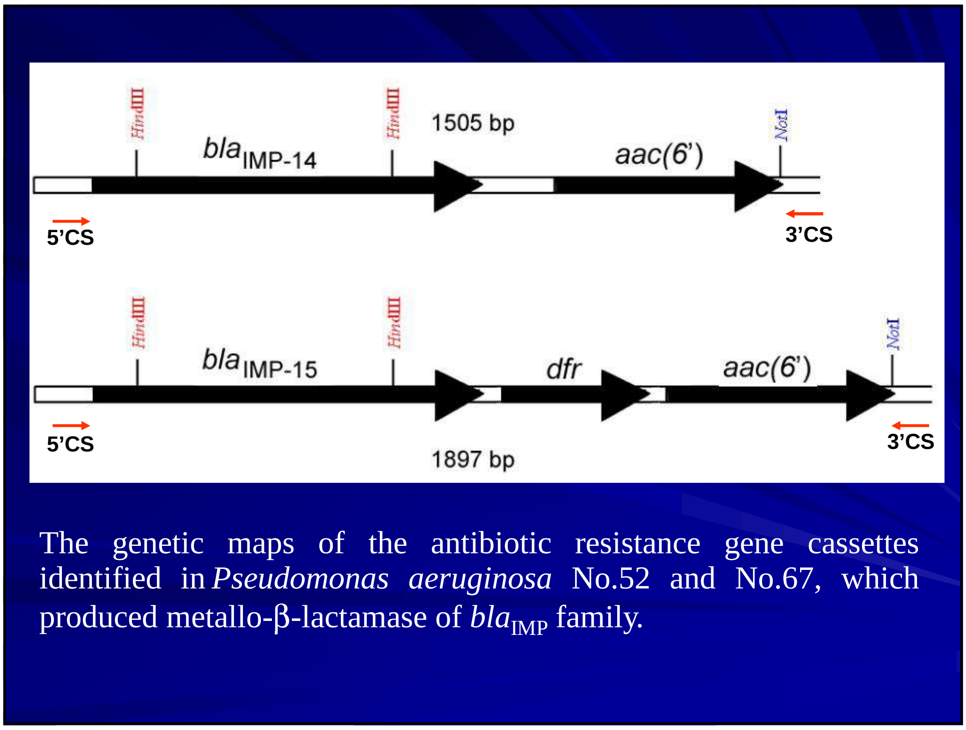
Resistance Genes Related to Integrons

Resistance gene	**Affected antibiotic**	**Coding for**
dfrA5, dfrA12	Sulfonamide/trimethoprim	Dihydrofolate reductase
cmlA	Chloramphenicol	Chloramphenicol transporter
qacEΔ1	Quaternary ammonium compound	Quaternary ammonium compound resistance
sul 1	Sulfonamide	Dihydropteroate synthase
qnrA	Fluoroquinolone	Fluoroquinolone protective protein
lnuF	Lincosamide	Lincosamide nucleotidyl transferase

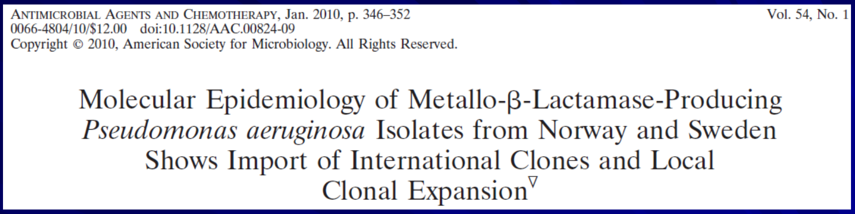


RAPD: *Pseudomonas aeruginosa*

Randomly Amplified Polymorphic DNA (RAPD) PCR amplification using single 10-mer primer.

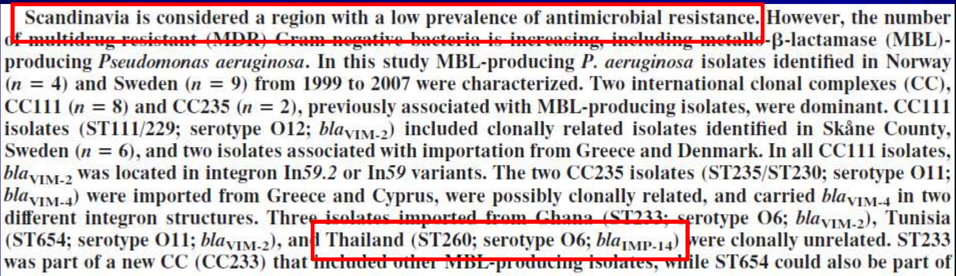


The genetic maps of the antibiotic resistance gene cassettes identified in *Pseudomonas aeruginosa* No.52 and No.67, which produced metallo-β-lactamase of bla_{IMP} family.



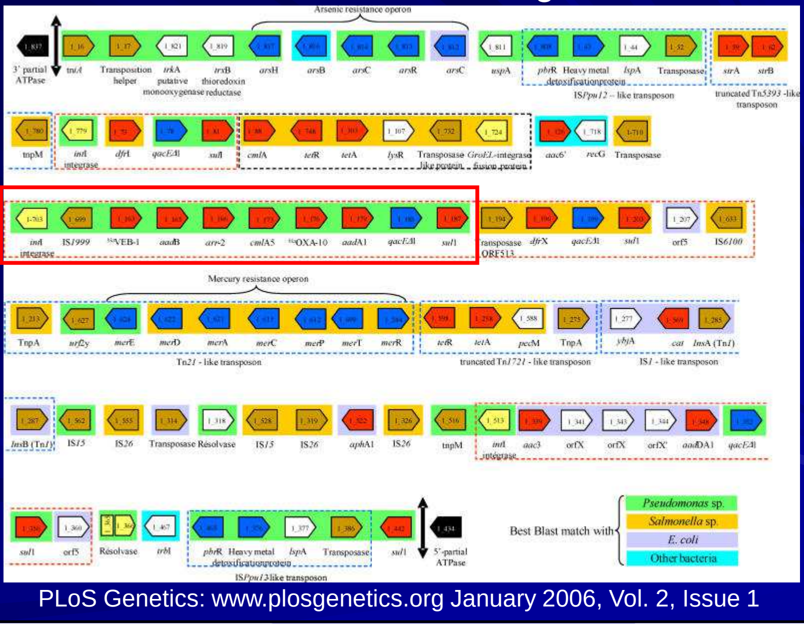
ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2010, p. 346–352 Vol. 54, No. 1
0066-4804/10/$12.00 doi:10.1128/AAC.00824-09

Molecular Epidemiology of Metallo-β-Lactamase-Producing *Pseudomonas aeruginosa* Isolates from Norway and Sweden Shows Import of International Clones and Local Clonal Expansion$^{\nabla}$



Scandinavia is considered a region with a low prevalence of antimicrobial resistance. However, the number of multidrug-resistant (MDR) Gram-negative bacteria is increasing, including metallo-β-lactamase (MBL)-producing *Pseudomonas aeruginosa*. In this study MBL-producing *P. aeruginosa* isolates identified in Norway (n = 4) and Sweden (n = 9) from 1999 to 2007 were characterized. Two international clonal complexes (CC), CC111 (n = 8) and CC235 (n = 2), previously associated with MBL-producing isolates, were dominant. CC111 isolates (ST111/229; serotype O12; *bla*$_{\text{VIM-2}}$) included clonally related isolates identified in Skåne County, Sweden (n = 6), and two isolates associated with importation from Greece and Denmark. In all CC111 isolates, *bla*$_{\text{VIM-2}}$ was located in integron In*59.2* or In*59* variants. The two CC235 isolates (ST235/ST230; serotype O11; *bla*$_{\text{VIM-4}}$) were imported from Greece and Cyprus, were possibly clonally related, and carried *bla*$_{\text{VIM-4}}$ in two different integron structures. Three isolates imported from Ghana (ST233; serotype O6; *bla*$_{\text{VIM-2}}$), Tunisia (ST654; serotype O11; *bla*$_{\text{VIM-2}}$), and Thailand (ST260; serotype O6; *bla*$_{\text{IMP-14}}$) were clonally unrelated. ST233 was part of a new CC (CC233) that included other MBL-producing isolates, while ST654 could also be part of a new CC associated with MBL producers. In the isolates imported from Ghana and Tunisia, *bla*$_{\text{VIM-2}}$ was part

86 kb Resistance Island *Acinetobacter baumannii* Total of 45 resistance genes



Best Blast match with
- *Pseudomonas* sp.
- *Salmonella* sp.
- *E. coli*
- Other bacteria

PLoS Genetics: www.plosgenetics.org January 2006, Vol. 2, Issue 1

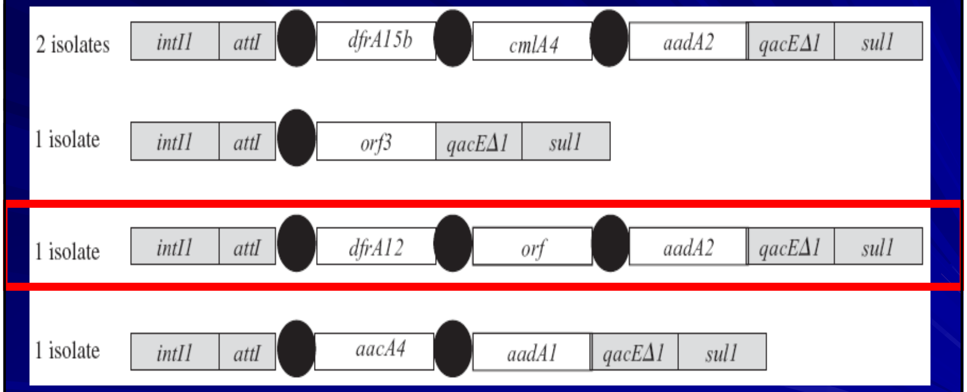
Mechanism of antimicrobial resistance in *Escherichia coli* and *Salmonella* from food animals in northern Thailand

Padungtod, P.[1], Tribuddharat, C.[2], Chuanchuen, R.[3]

[1] Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai, Thailand.
[2] Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University
[3] Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University

Widespread presence of *dfrA12* and its association with *dfrA12-aadA2* cassette in *Salmonella enterica* isolates from swine.
Padungtod P, Tribuddharat C, Chuanchuen R.
Southeast Asian J Trop Med Public Health. 2011 Nov;42(6):1471-6.

Various Class 1 Integrons



dfrA12 - aadA2 are common resistance gene cassettes in *Salmonella*- Taiwan, Ethiopia

Prevalence of *E. coli* and *Salmonella* spp. in sealed packages of chicken meat from supermarkets in Thailand

	Prevalence	Integrase (*intI1*)
E. coli	53% (106/200)	37.74% (40/106)
Salmonella	18.67% (14/75)	42.86% (6/14)

Jpn. J. Infect. Dis., 65, 527-534, 2012

Original Article

Molecular Characterization of Antibiotic-Resistant Bacteria in Contaminated Chicken Meat Sold at Supermarkets in Bangkok, Thailand

Chaiyaporn Chaisatit[1], Chanwit Tribuddharat[1]*, Chaiwat Pulsrikarn[2], and Surang Dejsirilert[2]

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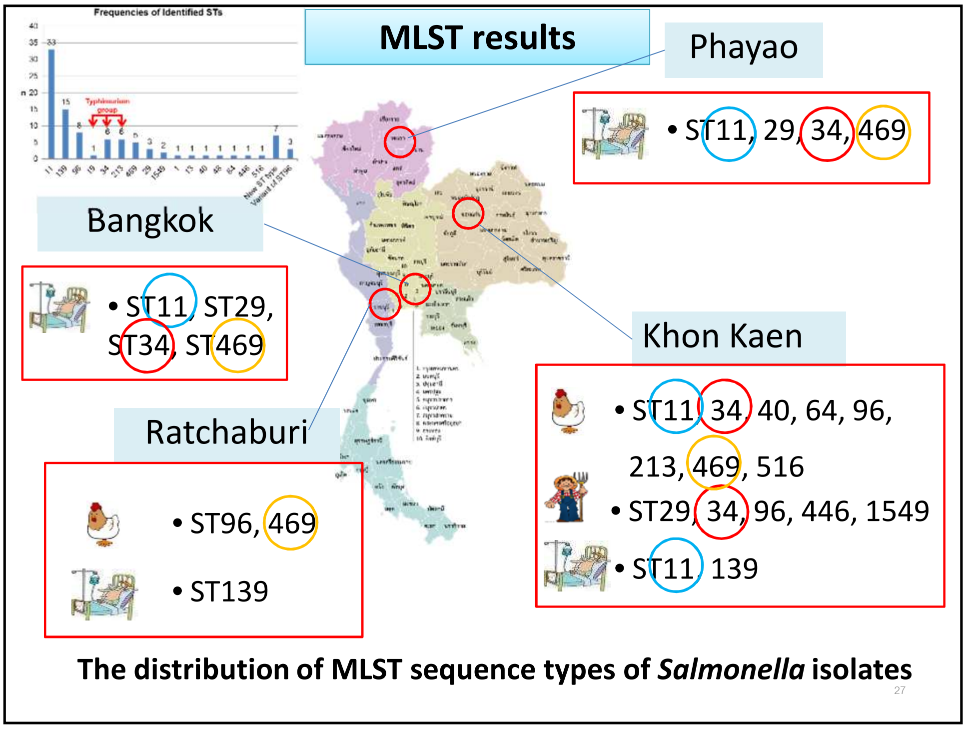
	intI1	5'CS-3'CS	Resistance cassette (s)	Resistance phenotype	Accession number	Dot blot
E9 (*E. coli*)	**+**	**1 kb**	***aadA23***	**AM, CIP, LEV, GM**	**FN252409**	**+**
E9 (*E. coli*)	**+**	**700 bp**	***dfrA5***	**AM, CIP, LEV, GM**	**GU562437**	**+**
E12 (*E. coli*)	**+**	**850 bp**	***aadA4***	**CIP, LEV, GM, SXT**	**FQ482074**	**+**
E17 (*E. coli*)	**-**	**500 bp**	***lnuF***	**AM, CF, GM**	**EU118119**	**+**
Sal130 (*S.* Kedougou)	**-**	**650 bp**	***∆aadA2*, *lnuF***	**AM, GM**	**EU118119**	**-**
Sal162 (*S.* Kedougou)	**+**	**1 kb**	***aadA22***	**AM, GM, TE**	**AB434537**	**+**



MLST results

Phayao

- ST11, 29, 34, 469

Bangkok

- ST11, ST29, ST34, ST469

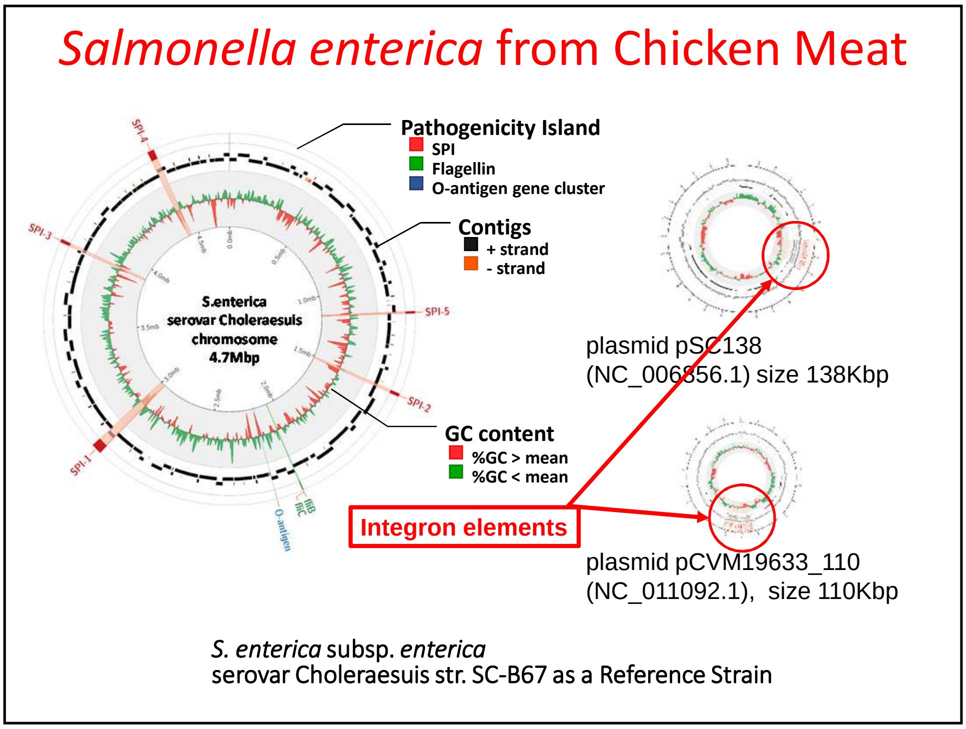
Khon Kaen

- ST11, 34, 40, 64, 96, 213, 469, 516
- ST29, 34, 96, 446, 1549
- ST11, 139

Ratchaburi

- ST96, 469
- ST139

The distribution of MLST sequence types of *Salmonella* isolates



Salmonella enterica from Chicken Meat

Pathogenicity Island
- 🟥 SPI
- 🟩 Flagellin
- 🟦 O-antigen gene cluster

Contigs
- ⬛ + strand
- 🟧 - strand

GC content
- 🟥 %GC > mean
- 🟩 %GC < mean

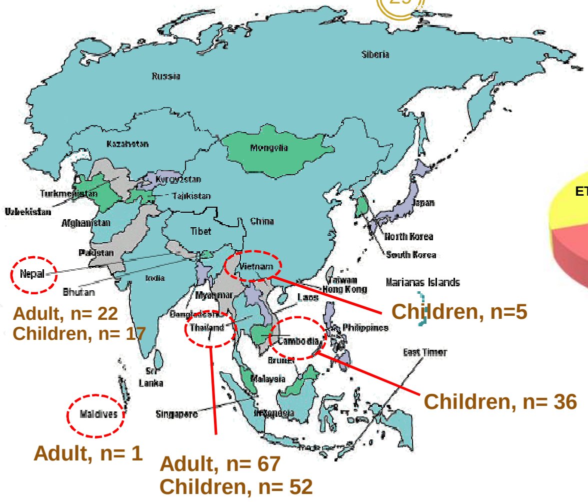
Integron elements

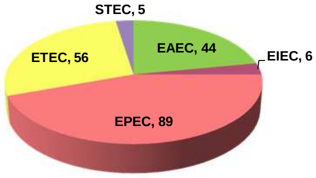
plasmid pSC138 (NC_006856.1) size 138Kbp

plasmid pCVM19633_110 (NC_011092.1), size 110Kbp

S. enterica subsp. *enterica*
serovar Choleraesuis str. SC-B67 as a Reference Strain

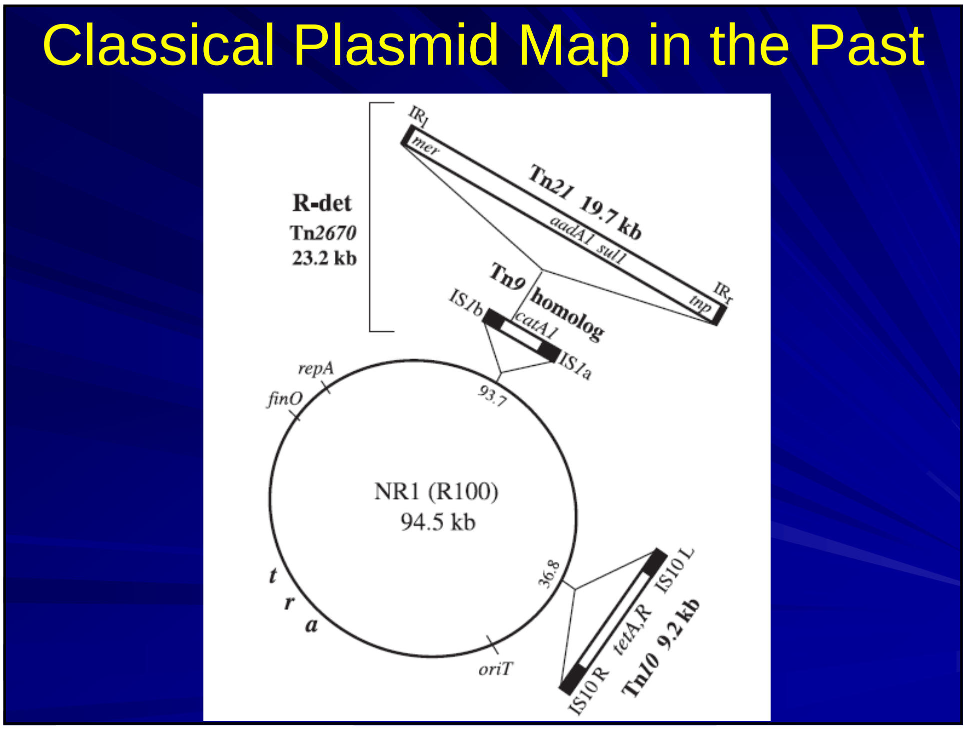
Enteropathogenic *E. coli* (Traveler's diarrhea)



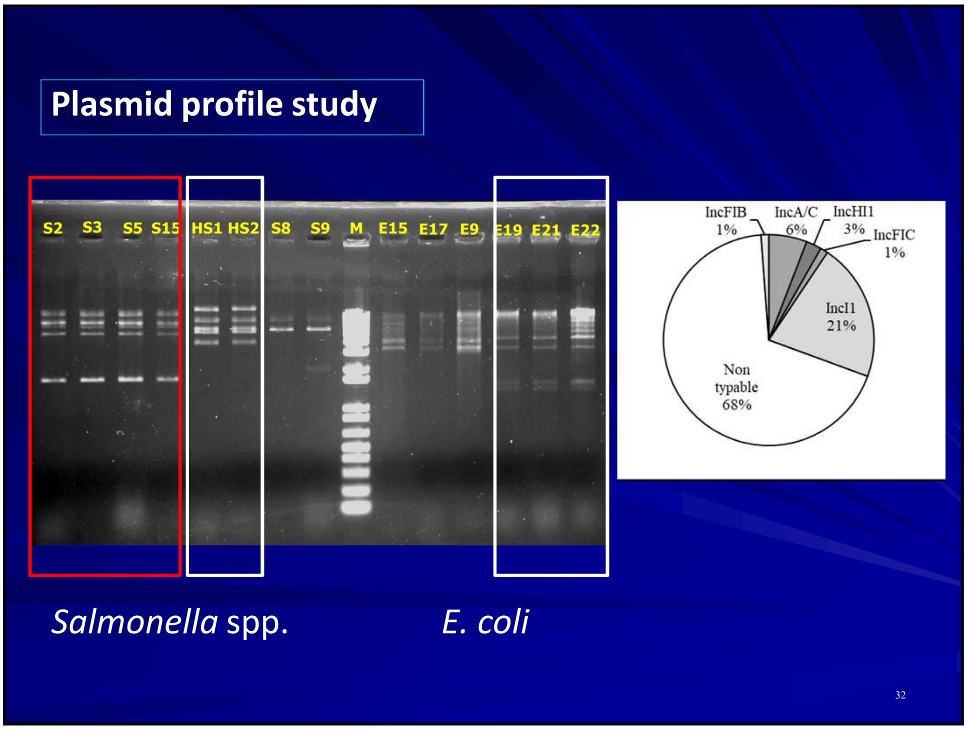


Investigation of possible outbreak and persistent clone

Isolate	Symptom	Pathotype	Year	Status	Age (yr)	Age (mo)	Nationality	Location	Integron	Genetic Type			
										ST cplx	ST	ERIC PCR	Plasmid profile
EA033	Yes	ETEC	2002	Adult	NA	NA	American	Sa Kaeo	neg	None	182	E24	P23
EA031	Yes	ETEC	2002	Adult	NA	NA	American	Sa Kaeo	neg	None	182	E24	P34
EA032	Yes	ETEC	2002	Adult	NA	NA	American	Sa Kaeo	neg	None	182	E24	P34
EC163	Yes	ETEC	2002	Children	0	4	Karen	Sankhlaburi	pos	None	182	E24	P23
EC177	Yes	ETEC	2002	Children	4	0	Thai	Sankhlaburi	neg	None	182	E24	P24
ET084	Yes	ETEC	2002	Adult	45	NA	European	Nepal	neg	None	182	E24	P32
ET083	Yes	ETEC	2002	Adult	26	NA	New Zealander	Nepal	neg	None	182	E24	P25
EA035	Yes	ETEC	2004	Adult	NA	NA	American	Korat	neg	None	182	E24	P34
ET082	Yes	ETEC	2001	Adult	24	NA	European	Nepal	neg	None	182	E24	P34
ET086	Yes	ETEC	2003	Adult	61	NA	Japanese	Nepal	neg	None	182	E24	P34
EC169	Yes	ETEC	2007	Children	1	6	Nepalese	Nepal	neg	None	182	E24	P34
EC183	Yes	ETEC	2004	Children	0	3	Cambodian	Cambodia	neg	None	182	E24	P34
EC172	No	ETEC	2008	Children	3	11	Nepalese	Nepal	neg	None	182	E24	P32
EC199	Yes	ETEC	2002	Children	4	1	Vietnamese	Vietnam	neg	None	182	E24	P32



Classical Plasmid Map in the Past



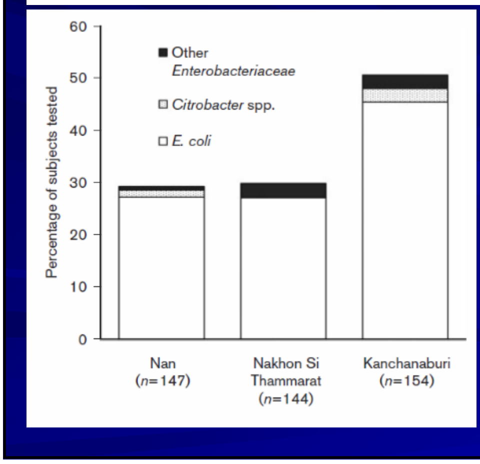
Plasmid profile study

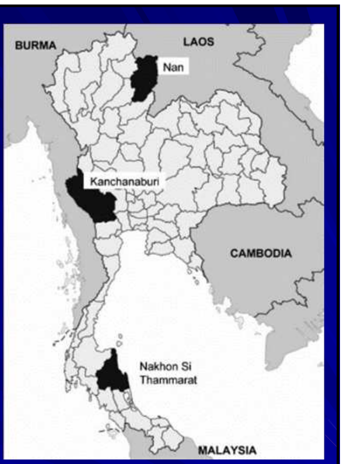
Salmonella spp.

E. coli

Analysis of risk factors for a high prevalence of extended-spectrum β-lactamase-producing *Enterobacteriaceae* in asymptomatic individuals in rural Thailand

Ulzii-Orshikh Luvsansharav,[1] Itaru Hirai,[1,2] Marie Niki,[1] Tadahiro Sasaki,[1] Kiyoko Makimoto,[3] Chalit Komalamisra,[4] Wanna Maipanich,[4] Teera Kusolsuk,[4] Surapol Sa-Nguankiat,[4] Somchit Pubampen[4] and Yoshimasa Yamamoto[1,2]





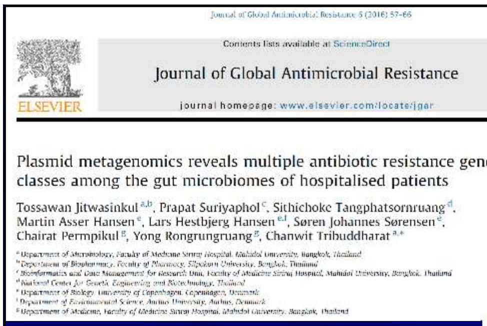
Journal of Medical Microbiology (2011), 60, 619–624

Prevalence of and risk factors associated with faecal carriage of CTX-M β-lactamase-producing Enterobacteriaceae in rural Thai communities

Ulzii-Orshikh Luvsansharav[1], Itaru Hirai[1], Arisa Nakata[1], Kaori Imura[1], Kou Yamauchi[1], Marie Niki[1], Chalit Komalamisra[2], Teera Kusolsuk[2] and Yoshimasa Yamamoto[1,3]*

District	Participants	ESBL phenotype[a]		CTX-M gene[b]	
Thong Pha Phum	232	148	63.8%	133	57.3%
Tha Maka	185	141	76.2%	141	76.2%
Total	417	289	69.3%	274	65.7%

J Antimicrob Chemother 2012; 67: 1769–1774



Journal of Global Antimicrobial Resistance 5 (2016) 57–66

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journal homepage: www.elsevier.com/locate/jgar

Plasmid metagenomics reveals multiple antibiotic resistance gene classes among the gut microbiomes of hospitalised patients

Tossawan Jitwasinkul[a,b], Prapat Suriyaphol[c], Sithichoke Tangphatsornruang[d], Martin Asser Hansen[e], Lars Hestbjerg Hansen[e,f], Søren Johannes Sørensen[e], Chairat Permpikul[g], Yong Rongrungruang[g], Chanwit Tribuddharat[a,*]

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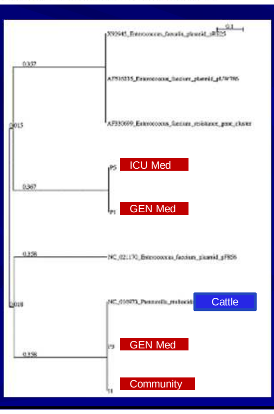
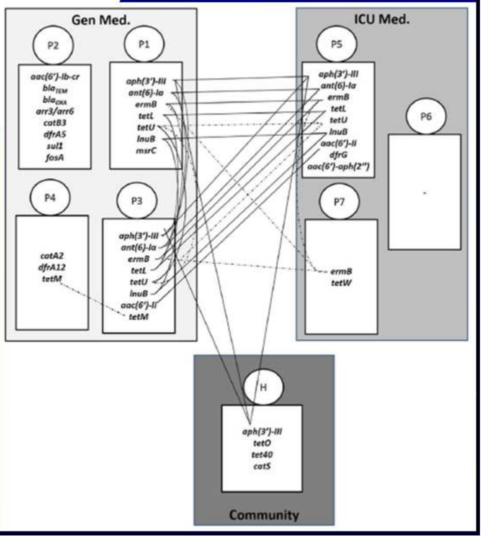




Eur J Clin Microbiol Infect Dis
DOI 10.1007/s10096-010-1031-y

August 2010

Colonisation with *Escherichia coli* resistant to "critically important" antibiotics: a high risk for international travellers

K. Kennedy · P. Collignon

Antibiotic	Percentage (95% CI) of travellers with resistant *E. coli* pre-travel	Percentage (95% CI) of travellers with resistant *E. coli* post-travel
Ciprofloxacin	3.9 (1.2–10.0)	33.3 (24.9–43.0)^{a}
Gentamicin	7.8 (3.8–14.9)	40.2 (31.2–49.9)^{a}
3GC	2.0 (0.1–7.3)	25.5 (18.0–34.8)^{a}
ESBL-positive	2. 0 (0.1–7.3)	21.6 (14.6–30.6)^{a}
Any antibiotic	7.8 (3.8–14.9)	49.0 (39.5–58.6)^{a}

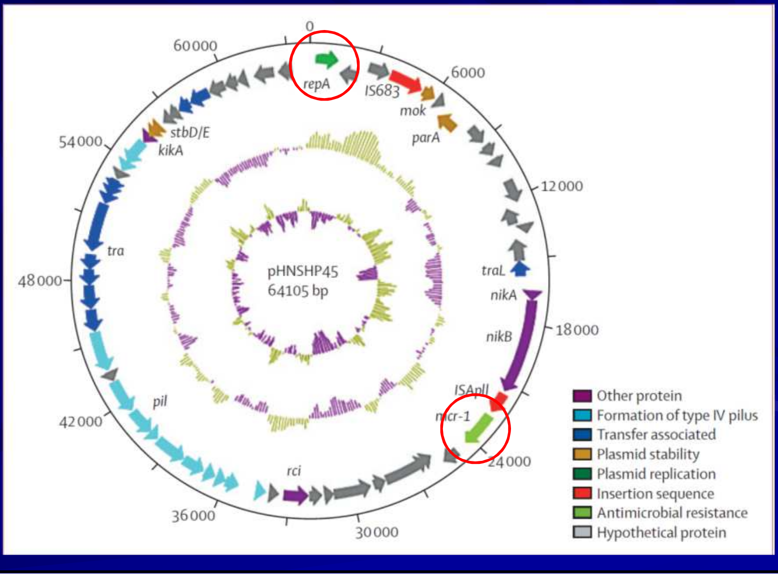
Plasmid-borne Polymyxin Resistance: MCR-1

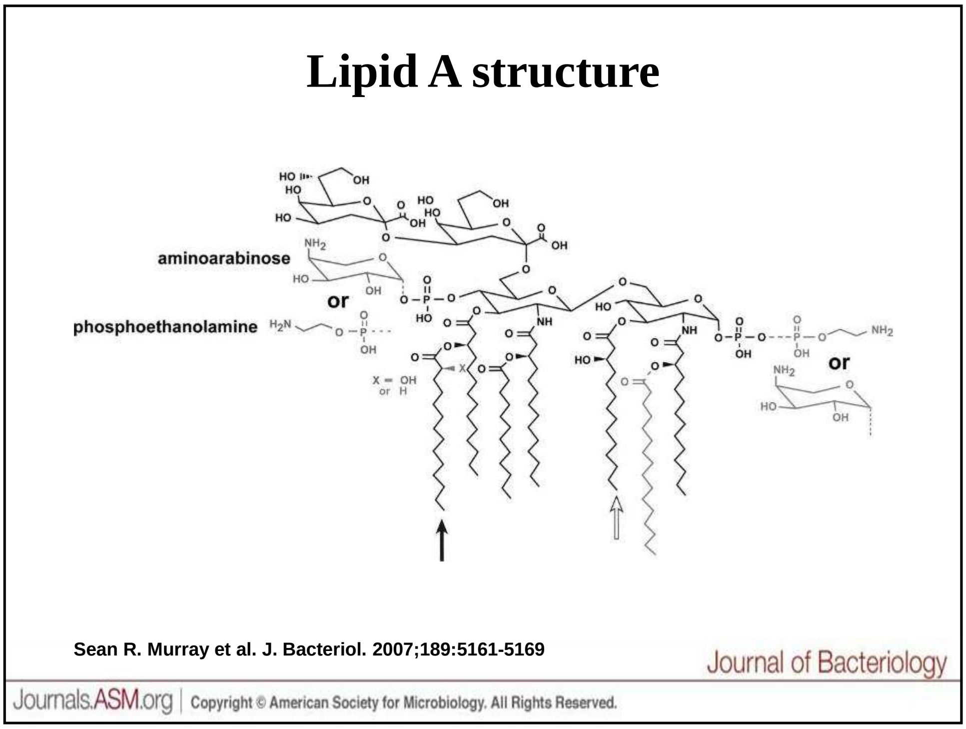
Mobile colistin resistance-1: November 2015

Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Liu, Y.-Y., Wang, Y., Walsh, T. R., Yi, L.-X., Zhang, R., Spencer, J., et al.

- ❑ MCR-1, a phosphoethanolamine transferase enzyme, resulting in the addition of phosphoethanolamine to lipid A
- ❑ Found in Enterobacteriaceae and *P. aeruginosa*
- ❑ *mcr-1 has been reported on all continents from human, animal, and environmental sources*
- Skov RL, Monnet DL. 2016. Plasmid-mediated colistin resistance (mcr-1 gene): three months later, the story unfolds. Euro Surveill 21.

Structure of plasmid pHNSHP45 carrying *mcr-1* from *Escherichia coli* strain SHP45

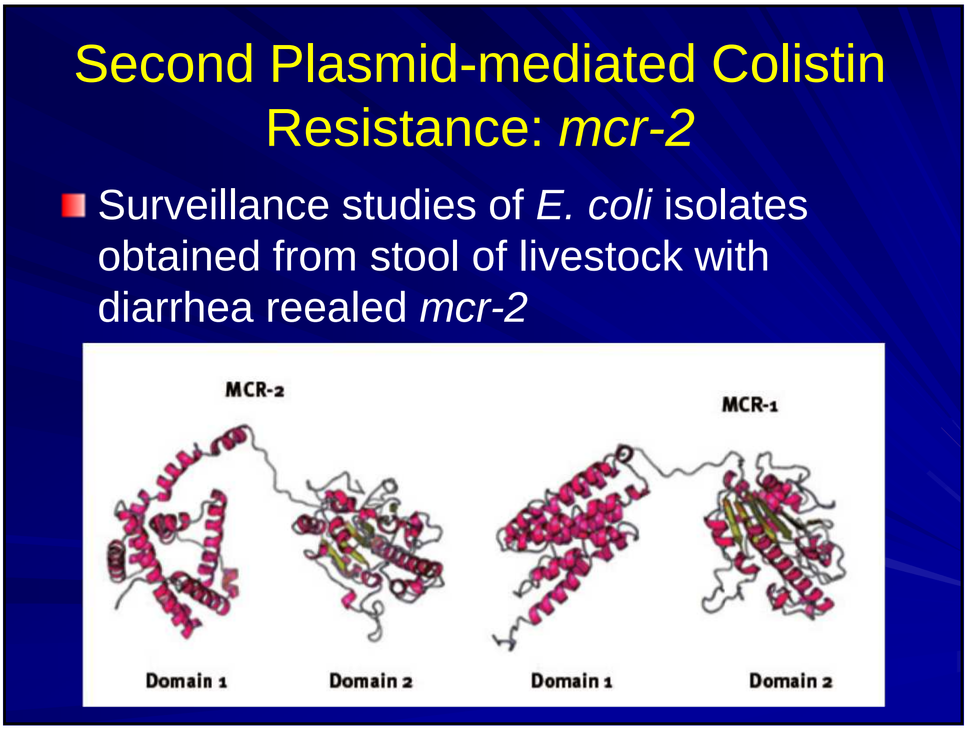




Lipid A structure

Sean R. Murray et al. J. Bacteriol. 2007;189:5161-5169

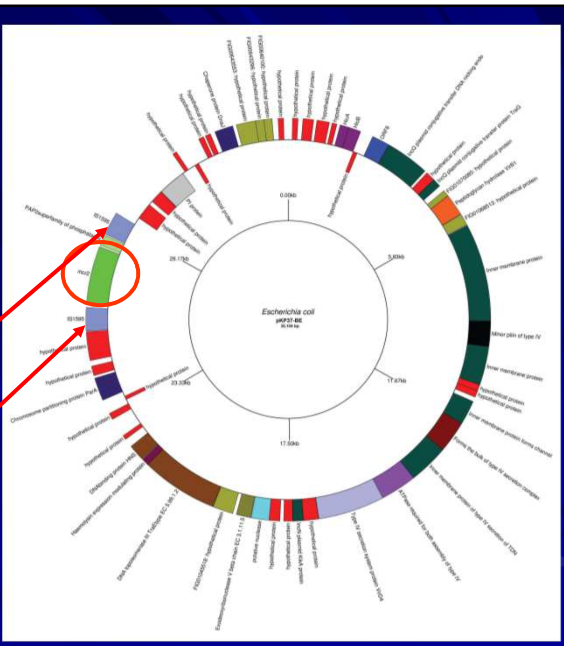
Journal of Bacteriology



Second Plasmid-mediated Colistin Resistance: *mcr-2*

- Surveillance studies of *E. coli* isolates obtained from stool of livestock with diarrhea reealed *mcr-2*

IncX4 Plasmid 35,104 bp

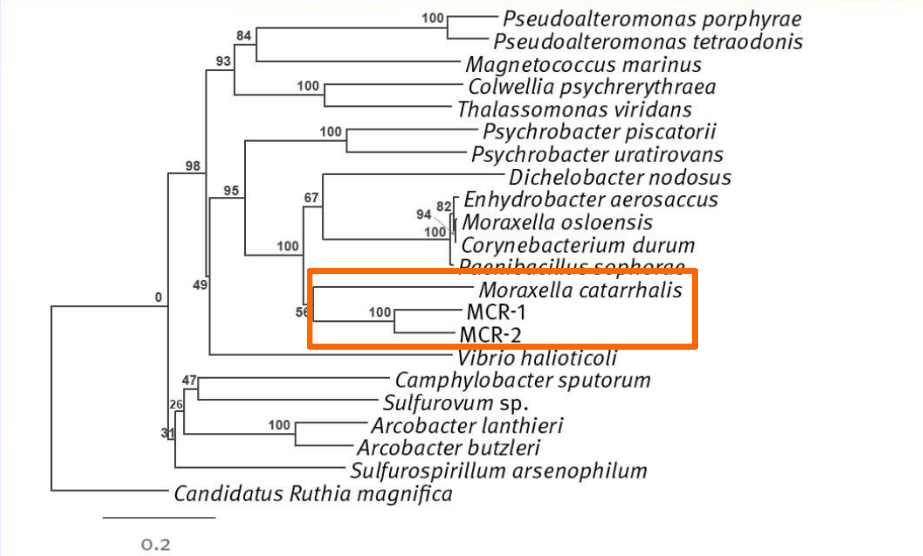


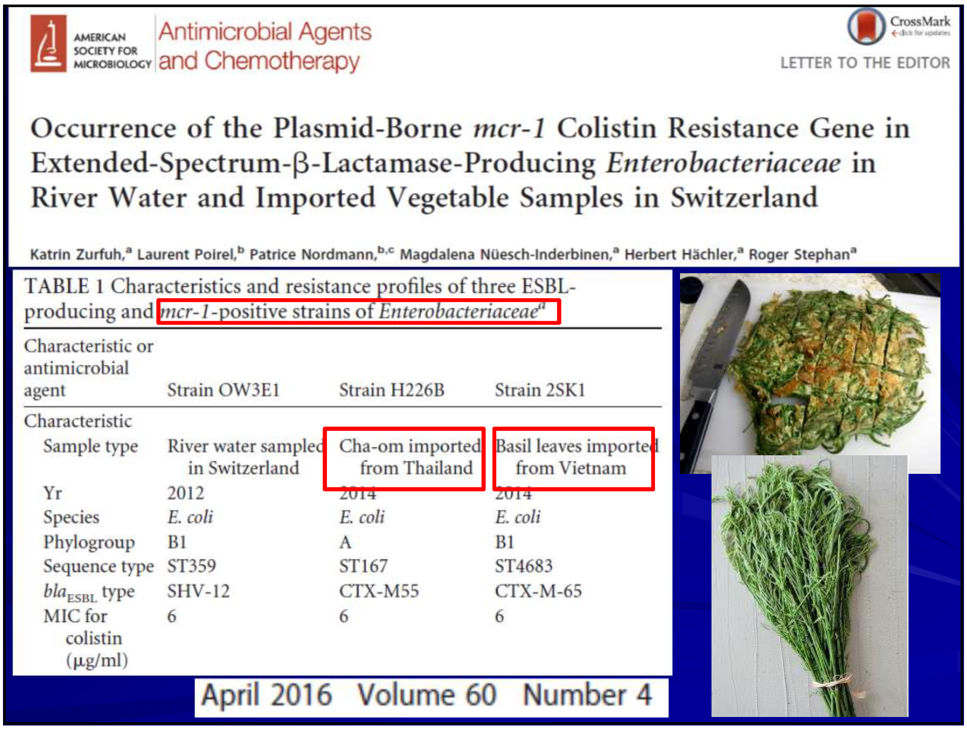
mcr-2-pap2

IS*1595*

Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, et al. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. Euro Surveill. 2016;21(27)

Phylogenetic analysis: MCR-2 protein





AMERICAN SOCIETY FOR MICROBIOLOGY Antimicrobial Agents and Chemotherapy

LETTER TO THE EDITOR

Occurrence of the Plasmid-Borne *mcr-1* Colistin Resistance Gene in Extended-Spectrum-β-Lactamase-Producing *Enterobacteriaceae* in River Water and Imported Vegetable Samples in Switzerland

Katrin Zurfuh,[a] Laurent Poirel,[b] Patrice Nordmann,[b,c] Magdalena Nüesch-Inderbinen,[a] Herbert Hächler,[a] Roger Stephan[a]

TABLE 1 Characteristics and resistance profiles of three ESBL-producing and *mcr-1*-positive strains of *Enterobacteriaceae*[a]

Characteristic or antimicrobial agent	Strain OW3E1	Strain H226B	Strain 2SK1
Characteristic			
Sample type	River water sampled in Switzerland	Cha-om imported from Thailand	Basil leaves imported from Vietnam
Yr	2012	2014	2014
Species	*E. coli*	*E. coli*	*E. coli*
Phylogroup	B1	A	B1
Sequence type	ST359	ST167	ST4683
bla$_{ESBL}$ type	SHV-12	CTX-M55	CTX-M-65
MIC for colistin (µg/ml)	6	6	6

April 2016 Volume 60 Number 4

Conclusions

- Tracing resistant bacteria is possible (and their resistance genes)
- History of housekeeping genes became antibiotic resistance genes
- Mobile genetic elements: transposons, insertion sequence element, and integron will disseminate the resistance genes
- More antibiotics used, more resistance genes
- Stop antibiotic use in livestock will help

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- National Institute of Infectious Diseases (NIID), Japan
- Roche
- AFRIMS