Antibiotic Resistance: What lie Beneath?

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Antibiotic Resistance: What lie Beneath?

- Introduction
- Genotype vs Phenotype
- Gram-positive resistance bacteria

  MRSA, VRE
- Gram-negative resistance bacteria

  Intrinsic resistance vs Plasmid resistance
- Beta-lactamase producing* (amC, ESBL, CRE)
- Summary : Q and A

No disclosure

Bad Bugs, No Drugs: No ESKAPE! An Update from the Infectious Diseases Society of America

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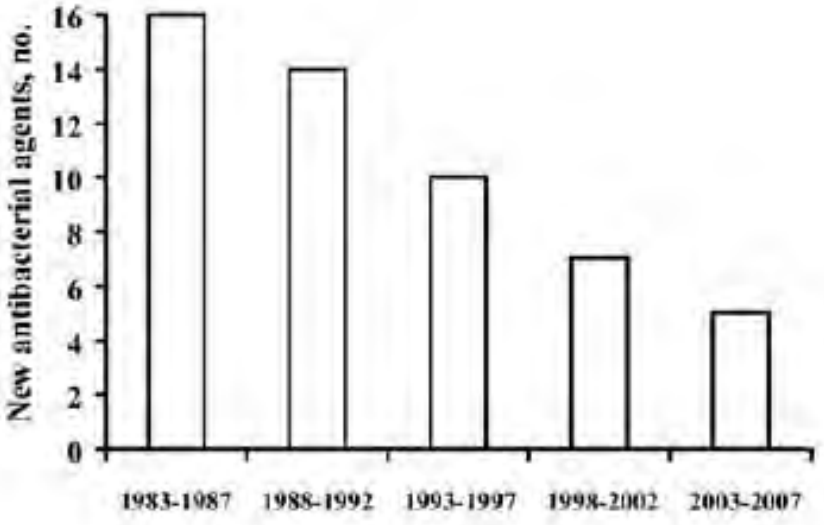

Figure 1. New antibacterial agents approved in the United States, 1983–2007, per 5-year period [2, 3].

Clinical Infectious Diseases 2009; 48:1–12

เชื้อจุลชีพดื้อยาที่มากขึ้น

ไม่มียาปฏิชีวนะใหม่

ไม่มีทางหนี?

ESKAPE

--- กลุ่มเชื้อจุลชีพดื้อยาหลัก

E. coli,

Salmonella, Stenotrophomonas, Serratia

Klebsiella spp.

Acinetobacter baunmannii

Pseudomonas aeruginosa, Proteus spp.

Enterobacter spp.

Centers for Disease Control and Prevention
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SEARCH

เชื้อจุลชีพดื้อยา (Multi-Drug Resistant Pathogens)

CDC A-Z INDEX

Antibiotic / Antimicrobial Resistance

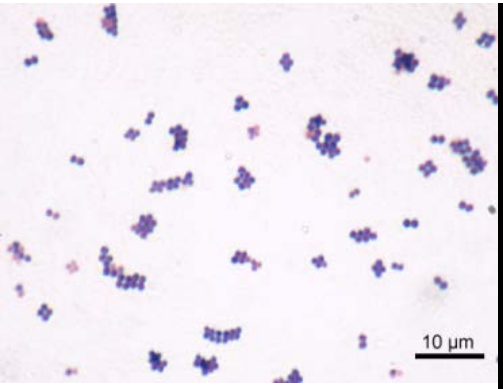
Antibiotics and similar drugs, together called antimicrobial agents, have been used for the last 70 years to treat patients who have infectious diseases. Since the 1940s, these drugs have greatly reduced illness and death from infectious diseases. However, these drugs have been used so widely and for so long that the infectious organisms the antibiotics are designed to kill have adapted to them, making the drugs less effective.

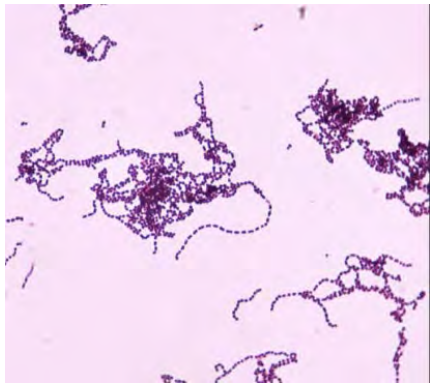
Each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die each year as a direct result of these infections.

Gram-positive bacteria

VRE (Vancomycin-resistant Enterococci)

MRSA (Methicillin-resistant *Staphylococcus aureus*)





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SEARCH

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Each year in the United States, at least 2 million people become infected with bacteria that are resistant to antibiotics and at least 23,000 people die each year as a direct result of these infections.

What is this MDR bug?

Gram-positive bacteria

VRE (Vancomycin-resistant Enterococci)

MRSA (Methicillin-resistant *Staphylococcus aureus*)

Gram-negative bacteria

- **Family Enterobactericeae (ESBL, CRE, AmpC)**

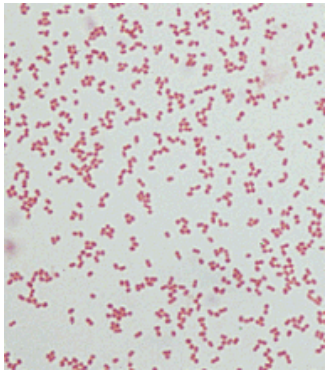
– *E. coli, Klebsiella, Enterobacter,*

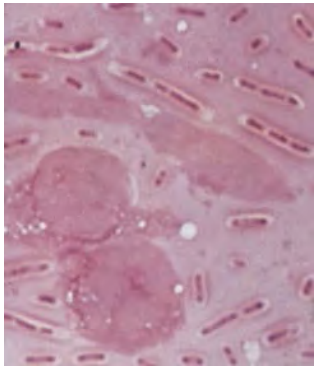
Proteus, Salmonella, Shigella, Citrobacter, Serratia

- **Nonfermenters** - ***Pseudomonase aeruginosa***

- Stenotrophomonas multocida

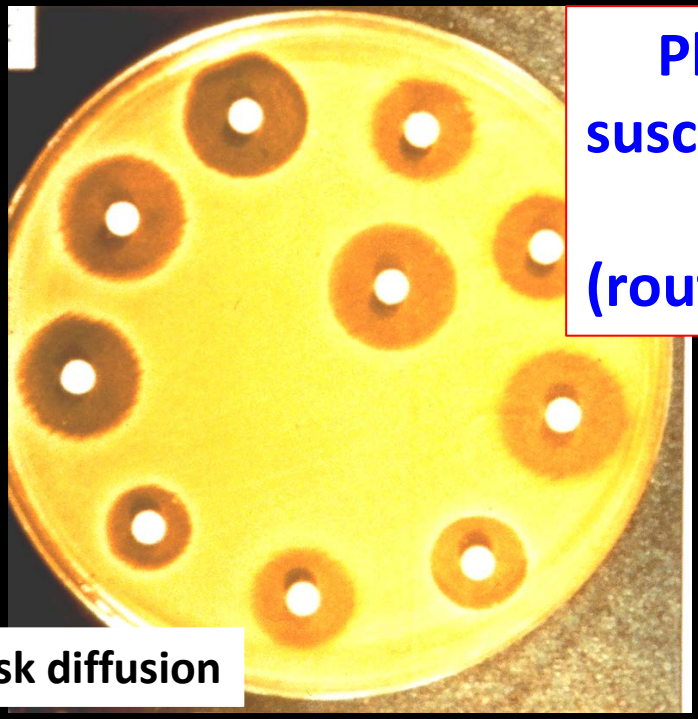
- Acinetobacter baumaunnii



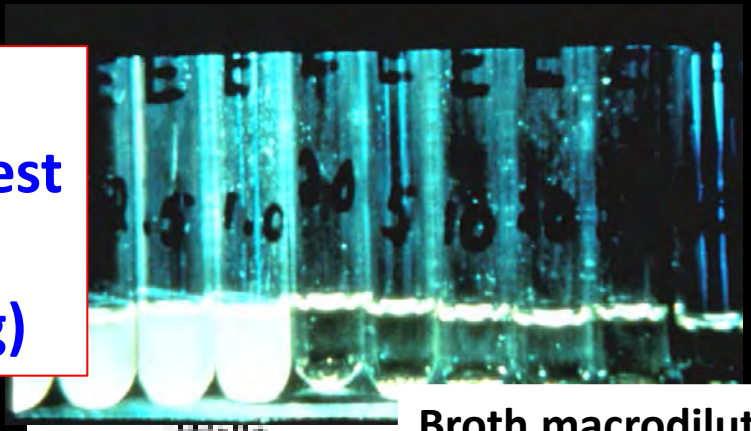


Phenotypic susceptibility test

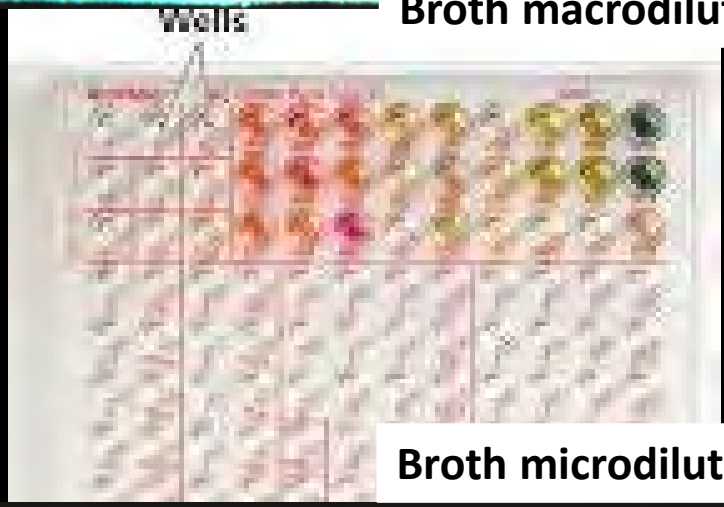
(routine testing)



Disk diffusion

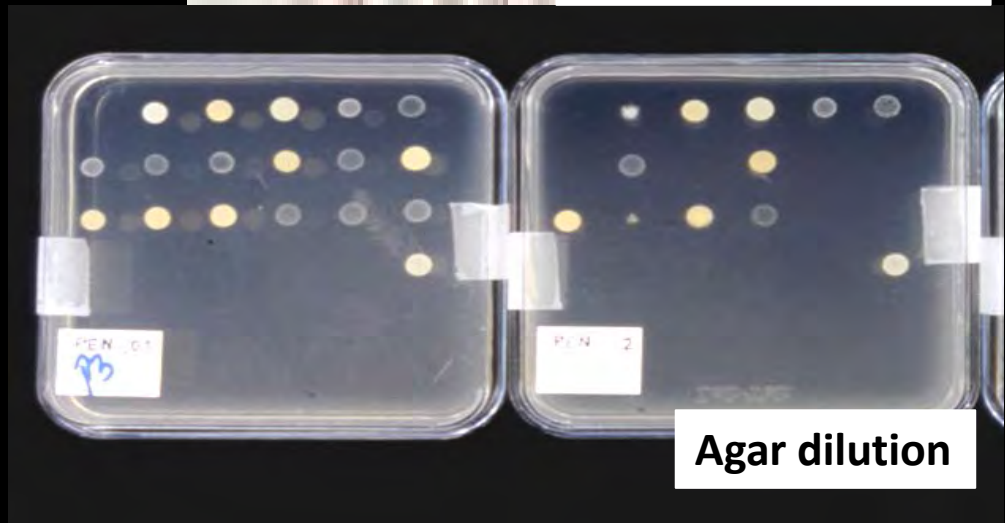


Broth macrodilution



Broth microdilution

E-test

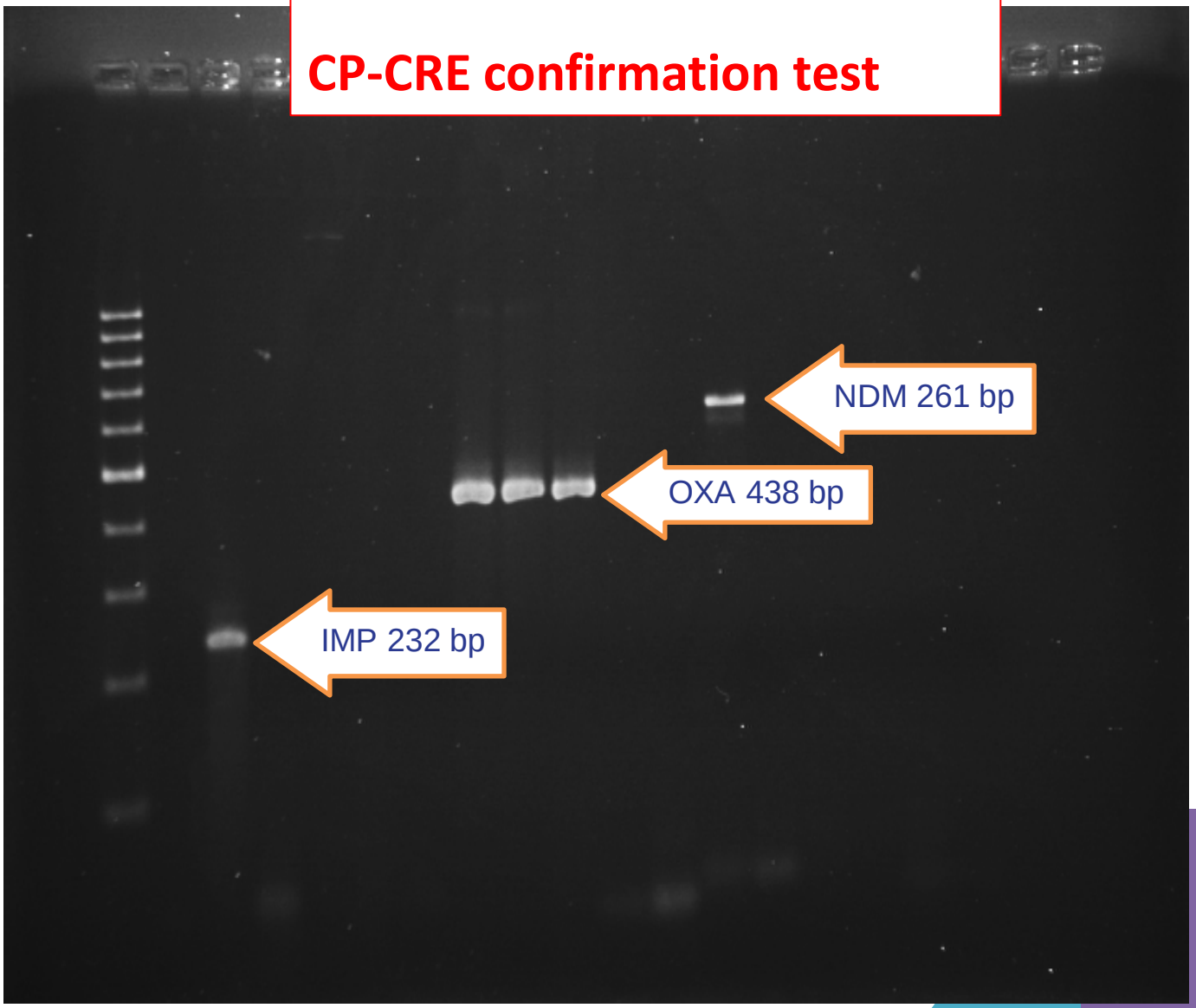


Agar dilution

ผลการทำ PCR

Genotypic /

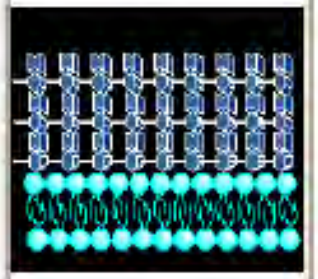
CP-CRE confirmation test

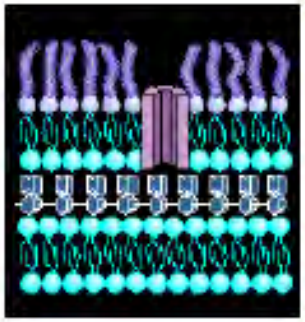


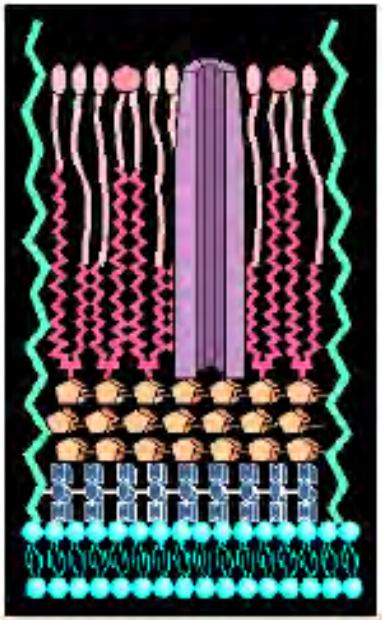
Comparison of gram-positive, gram-negative and mycobacterial cell walls

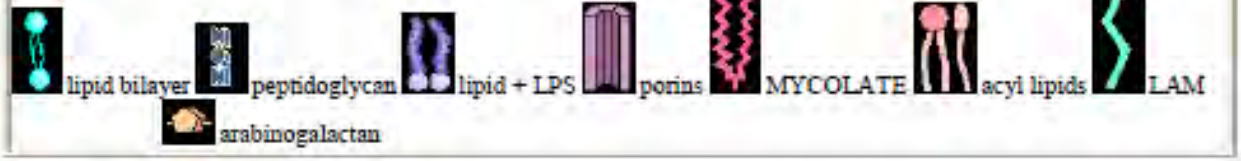
THE BACTERIAL CELL WALL

Gram-positive organisms	Gram-negative organisms	Mycobacteria
The lipid bilayer cell membrane of most of the Gram-positive bacteria is covered by a porous **peptidoglycan layer** which does not exclude most antimicrobial agents.	Gram-negative bacteria are surrounded by two membranes. The outer membrane functions as an efficient permeability barrier because it contains lipopolysaccharides (LPS) and porins.	Mycobacteria produce a thick **mycolate-rich** outer covering which functions as an exceptionally efficient barrier.







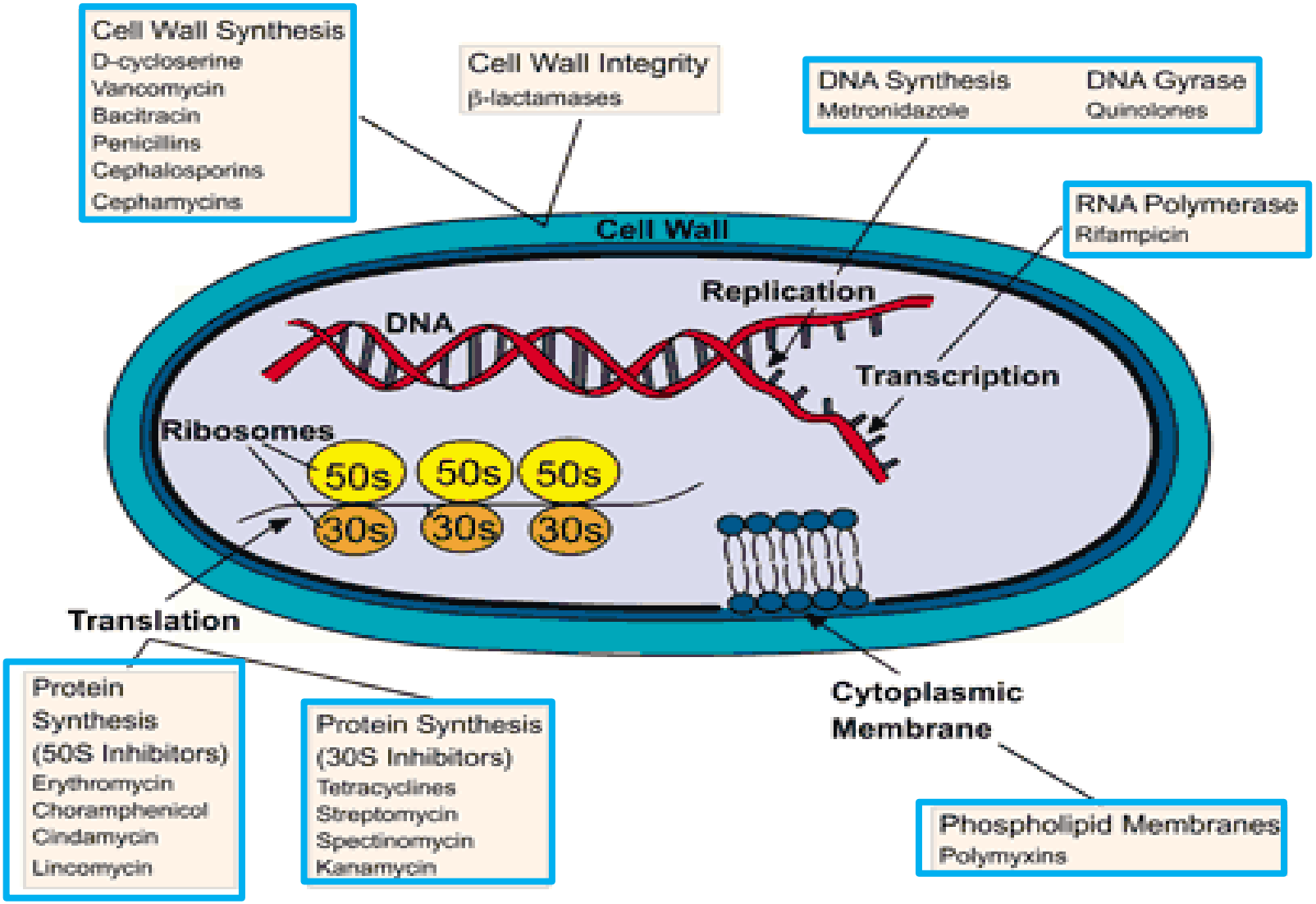


Antibiotics: Mechanism of action

Mechanism of action	Antimicrobial agents
Inhibition of cell wall synthesis *	Betalactams (penicillins, cephalosporins, aztreonam, carbapenem) vancomycin
Inhibition of bacterial protein synthesis #	Aminoglycosides, chloramphenicol, macrolides, tetracyclines, streptogramins, linezolid
Inhibition of nucleic synthesis *	Fluoroquinolones, rifampin
Inhibition of folic acid synthesis	Sulfonamides*, trimethoprim#, pyrimethamine
Disruption of cell membrane function *	Colistin Azole and polyene antifungal agents

Bacteriocidal* vs Bacteriostatic#

Site of Actions of Antimicrobial Agents



Active Surveillance MRSA

- **Recommended by SHEA/CDC**
  Legislated in some states in the US
- **Methods**

– Chromogenic agar media
– Molecular methods
• Variety of platforms
–Sensitivity:88-100%;
–Specificity :92-99 %
• Problems
–*mecA* dropouts
–Some MR-CoNs may test positive
–False negatives have also been reported due to emergent strains wi
unusual genotypes

S. aureus
= Coagulase test positive

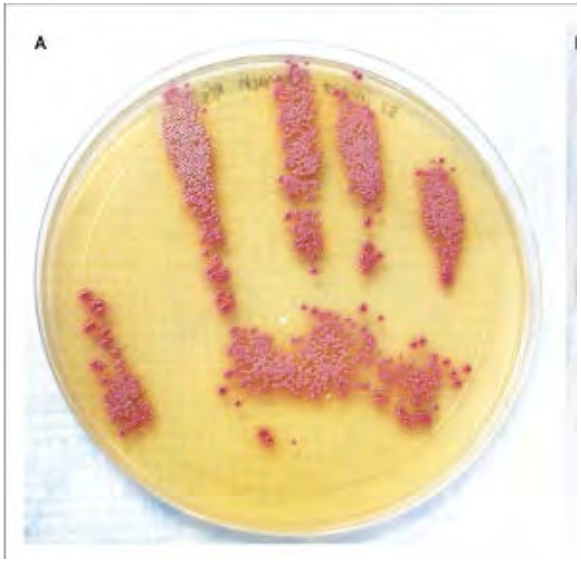
Muto CA, et. al. *Infect Control Hosp Epidemiol* 2003;24:362. Peterson LR, et al. *J Clin Microbiol* 2010;48:1661; Arbefeville SS, et al 2011; *J Clin Microbiol* 49:2996; Malhotra-Kumar et al 2010; *J Clin Microbiology* 48:4598

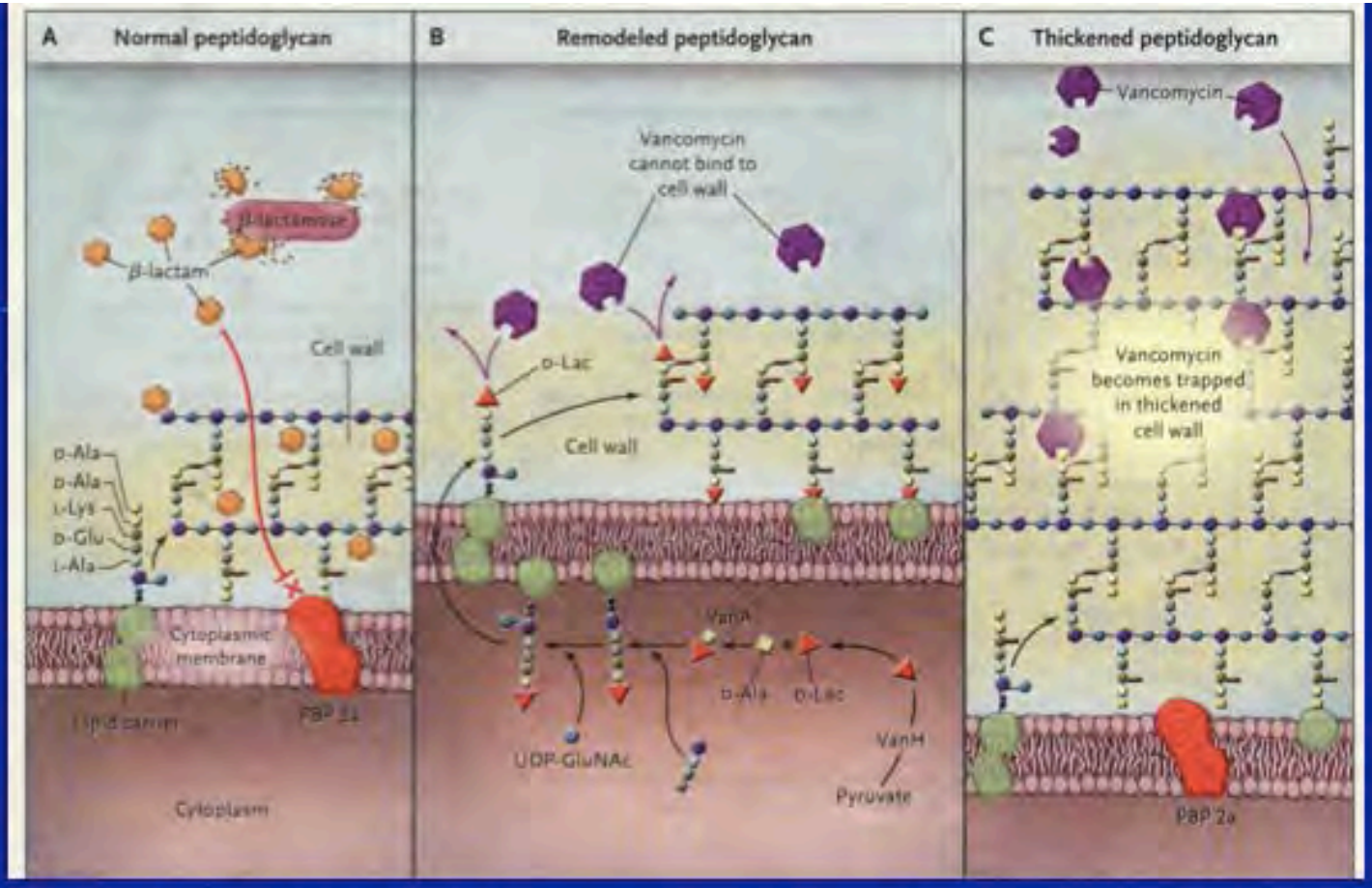
Staphylococcus aureus

- **Isolation procedure**
- 5% Sheep blood Agar (broth may enhance recovery)
- Incubation: 18-24 hr

Selective agars:

- CNA (Columbia Colistin-nalidixic acid agars)
- **Mannitol salt agar**
- Lipase-salt-mannitol agar (Remel)
- CHROMagar *Staph aureus* (CHROMagar,Paris, France)
- **BBL CHROMagar *Staph aureus**** (BD-Diagnostics)
- *S. aureus* ID (bioMerieux, France)





MRSA: Altered penicillin-binding protein (PBP2a) mecA gene

VRSA: D-lactate replaces D-alanine as the last amino acid of peptidoglycan precursors VanA

VISA: Thickened peptidoglycan layer traps vancomycin

NEJM-review, IDSA Guideline for MRSA treatment, Endocarditis treatment MSSA vs MRSA
Definition, Mechanism of resistance, CA-MRSA vs HA-MRSA (USA type, Scc type)

Vancomycin Resistance in *S. aureus*

Strain	Definition	Genetic event	Mechanism/ Significance
Pen Resistance		Penicillinase	Enzyme Modification
MRSA	Meth/Ox resistance	*mecA*	PBP2a ***Normal Cell wall***
Vanc suscept *S. aureus*-VSSA	**MIC ≤ 2 µg/mL**	-	
Vanco-intermediate *S. aureus* (VISA)	MIC 4-8 µg/mL	Unknown; ? *vraSR* & *graSR* mutation Cui *AAC* 2009; 53:1231	***-Thickened cell wall*** - increased vanco binding
Vanco-resistant *S. aureus* (VRSA)	MIC ≥ 16 µg/mL	**vanA from VR *E. faecalis***	***Remodeled Cell Wall*** D-ala-D-ala to D-ala-D-lactate

Vancomycin MIC creeping (MIC > 1) → more treatment failure

→ Vancomycin should not be avoided.

Use: Linezolid/ Daptomycin/ Ceftaroline

Endocarditis, Severe Pneumonia, Severe Skin infection, Osteomyelitis

Need to monitor Vancomycin MIC and vancomycin trough level (drug level)

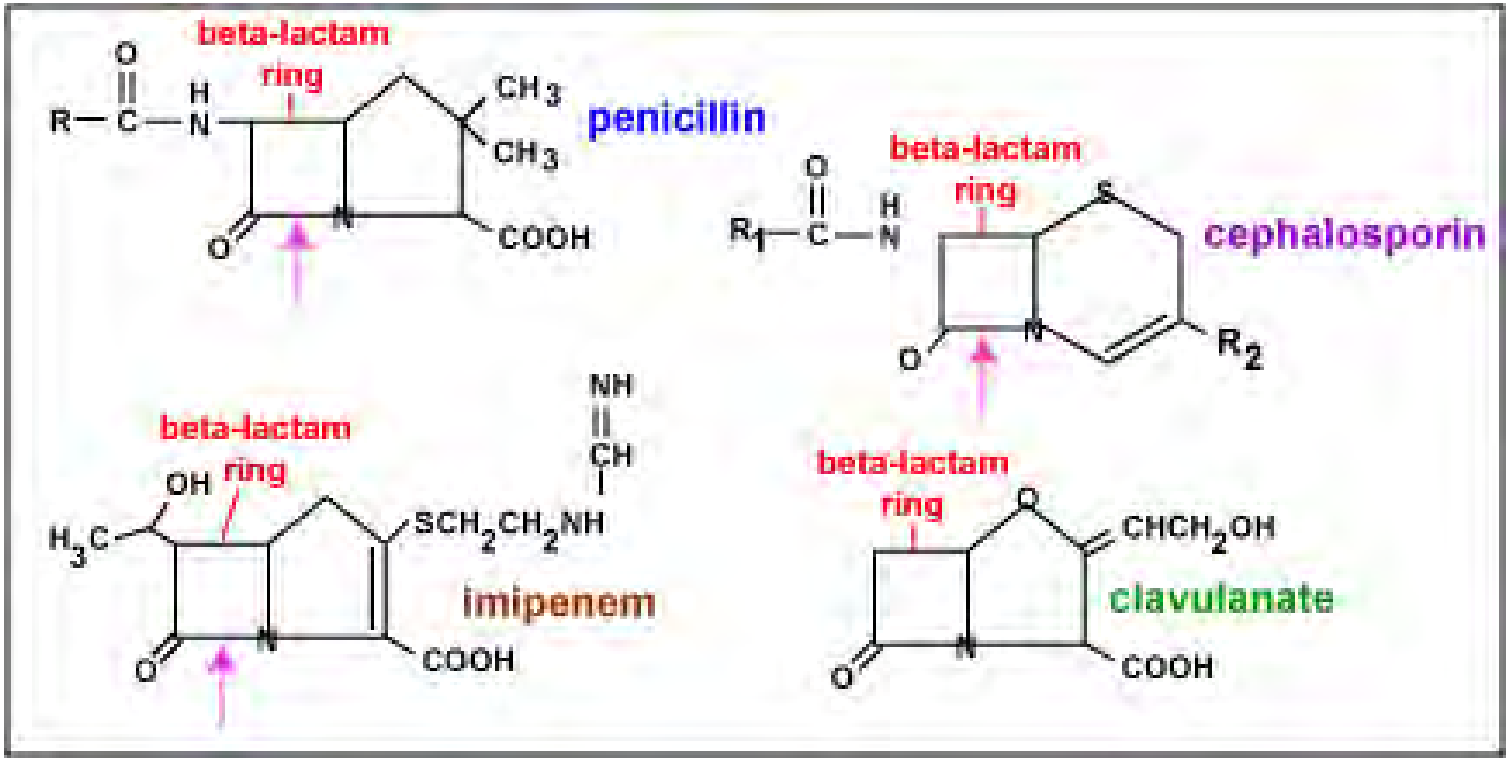
New Jan 2017 (CLSI: M100S27)

Table 2C. *Staphylococcus* spp. (Continued)

Test/Report Group	Antimicrobial Agent	Disk Content	Interpretive Categories and Zone Diameter Breakpoints (nearest whole mm) S	I	R	Interpretive Categories and MIC Breakpoints (µg/mL) S	I	R	Comments
GLYCOPEPTIDES (19) For *S. aureus*, vancomycin-susceptible isolates may become vancomycin intermediate during the course of prolonged therapy.									
B	Vancomycin (For *S. aureus*)	–	–	–	–	≤2	4–8	≥16	For use with *S. aureus*. (20) MIC tests should be performed to determine the susceptibility of all isolates of staphylococci to vancomycin. The disk test does not differentiate vancomycin-susceptible isolates of *S. aureus* from vancomycin-intermediate isolates, nor does the test differentiate among vancomycin-susceptible, -intermediate, and -resistant isolates of CoNS, all of which give similar size zones of inhibition. (21) Send any *S. aureus* for which the vancomycin is ≥ 8 µg/mL to a reference laboratory. See Appendix A. Also refer to Table 3G for *S. aureus*, Subchapter 3.13.1.7 in M07-A10, and Subchapter 3.9.1.7 in M02-A12.
B	Vancomycin (For CoNS)	–	–	–	–	≤4	8–16	≥32	For use with CoNS. See comment **(20)**. **(22)** Send any CoNS for which the vancomycin MIC is ≥ 32 µg/mL to a reference laboratory. See Appendix A. See also Subchapter 3.13.1.7 in M07-A10, and Subchapter 3.9.1.7 in M02-A12.
Inv.	Teicoplanin	–	–	–	–	≤8	16	≥32	
LIPOGLYCOPEPTIDES									
C	Oritavancin	–	–	–	–	≤0.12	–	–	See comment **(17)**.
C	Telavancin	–	–	–	–	≤0.12	–	–	See comment **(17)**.
LIPOPEPTIDES									
B	Daptomycin	–	–	–	–	≤1	–	–	**(23)** Daptomycin should not be reported for isolates from the respiratory tract.

MRSA: Vancomycin test = MIC only
No disk diffusion breakpoint

β – Lactams



Can we use carbapenem for MRSA?

D- test: Micro labs report should be….

Erythromycin-Resistance

Clindamycin-Resistance

(Macrolide-inducible clindamycin resistance)

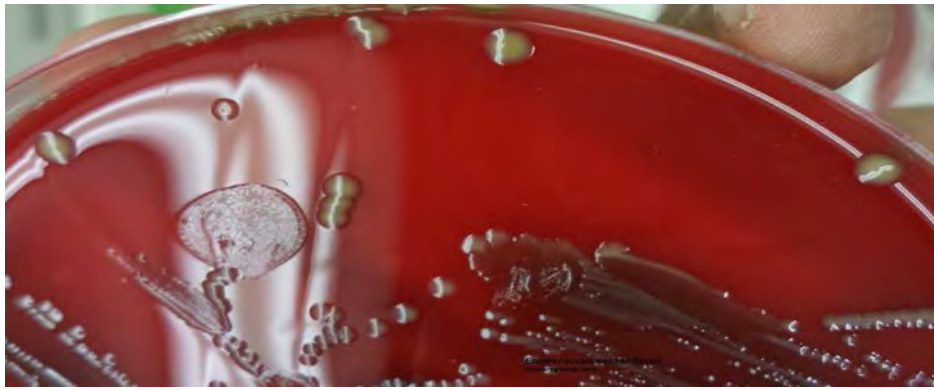
Active Surveillance
Vancomycin Resistant Enterococci (VRE)

Recommended by SHEA

– Active surveillance for high risk institutions

– Vigorous infection control practices

- Isolation of colonized patients
- Use of barrier precautions
- Hand hygiene
- Control antibiotic pressure



- **Methods**

–Stool culture

BHI with 6 µg/ml (or higher) of vancomycin

Chromogenic agars

Molecular methods are non-specific

Enteroccus spp

- Vancomycin-resistant enterococci (VRE): US
- 1st=1993(0.3%), 2006-2007= over 12%
- Increased mortality, liver transplant, HD,
- BMT patients (screening for VRE colonizations)

- *E. faecalis*: most common isolates (80-90%), VRE= 2%
- *E. faecium*: 5-10%, *VRE = 60-80%

- * VanA, VanB gene = Acquired resistance → Isolation

- *E. casseliflavus, E. gallinarum, E. raffinosus*…etc

Vol. 35, No. 6 www.cmnewsletter.com March 15, 2013

Current Rapid Screening Methods for Gastrointestinal Colonization of Vancomycin-Resistant Enterococci

J. Kristie Johnson, Ph.D, D(ABMM)[1,2] and Donna Cashara, BS, MT (ASCP),[2] [1]Department of Pathology University of Maryland School of Medicine, and [2]Microbiology Laboratory, University of Maryland Medical Center, Baltimore, Maryland

Table 1. Glycopeptide resistance in enteroccci

	Value							
Characteristic[a]	*vanA*	*vanB*[b]	*vanC*[b]	*vanD*[b]	*vanE*	*vanG*[b]	*vanL*	*vanM*
Type of resistance	Acquired	Acquired	Intrinsic	Acquired	Acquired	Acquired	Acquired	Acquired
MIC (μg/ml)								
Vancomycin	64–1,000	8–1,000	2–32	64–128	8–32	16	8	>256
Teicoplanin	6–512	0.5–1	0.5–1	4–64	0.5	0.5	8	0.75–48
Location of *van* gene	Plasmid, chromosome	Plasmid, chromosome	Chromosome	Chromosome	Chromosome	Chromosome	Unknown	Plasmid
Gene product	D-Ala-D-Lac[c]	D-Ala-D-Lac	D-Ala-D-Ser[d]	D-Ala-D-Lac	D-Ala-D-Ser	D-Ala-D-Ser	D-Ala-D-Ser	D-Ala-D-Lac

[a] Data from references 1, 20, 44, and 45.
[b] Subtypes exist: *vanB* (1 to 3), *vanC* (1 to 4), *vanD* (1 to 5), *vanG* (1 and 2).
[c] D-Alanine-D-lactate.
[d] D-Alanine-D-serine.

Mechanism of GN resistance

- **Enzymatic resistance**

  Non-enzymatic resistance

- **Acquired (plasmid/transferable resistance**

  Intrinsic (chromosomal) resistance

Phenotypic resistance

vs

Genotypic resistance

****Enzymatic : ESBLs/ CREs**

(hydrolytic enz)

betalactamase

(several bla genes)

Non-enzymatic

-Efflux pump

-Porin change

(oprD gene)

-Decreased

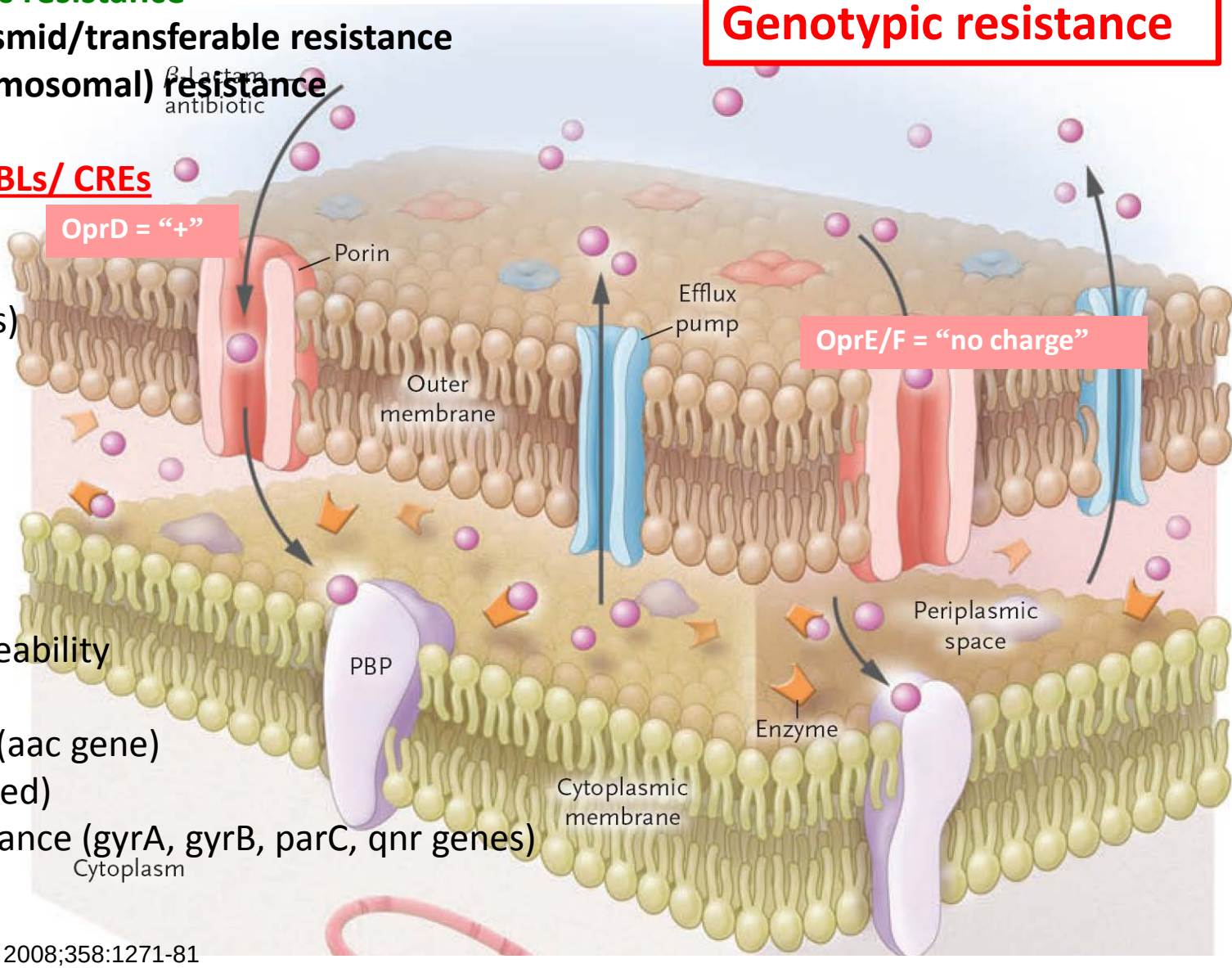
membrane permeability

(omp gene)

-Aminoglycoside (aac gene)

(target site changed)

-Quinolone resistance (gyrA, gyrB, parC, qnr genes)



AAC 1999;43(2):424-7 NEJM 2008;358:1271-81

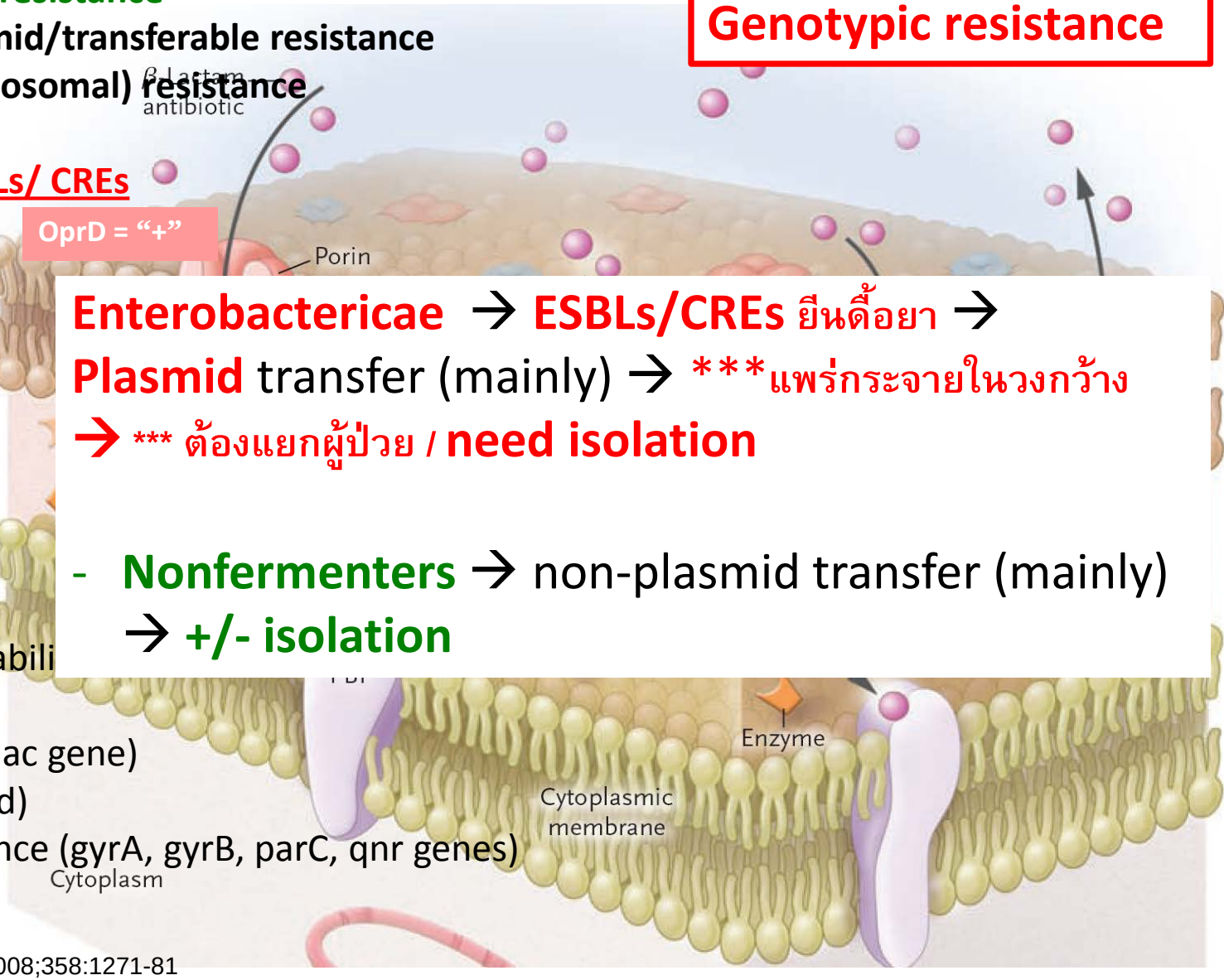
Mechanism of GN resistance

- **Enzymatic resistance**
  Non-enzymatic resistance
- **Acquired (plasmid/transferable resistance**
  Intrinsic (chromosomal) resistance

Phenotypic resistance
vs
Genotypic resistance

****Enzymatic : ESBLs/ CREs**
(hydrolytic enz)
betalactamase
(several bla genes)

Non-enzymatic
-Efflux pump
-Porin change
(oprD gene)
-Decreased
membrane permeabili
(omp gene)
-Aminoglycoside (aac gene)
(target site changed)
-Quinolone resistance (gyrA, gyrB, parC, qnr genes)



Enterobactericae → ESBLs/CREs ยีนดื้อยา → Plasmid transfer (mainly) → ***แพร่กระจายในวงกว้าง
→ *** ต้องแยกผู้ป่วย / **need isolation**

- **Nonfermenters** → non-plasmid transfer (mainly)
  → **+/- isolation**

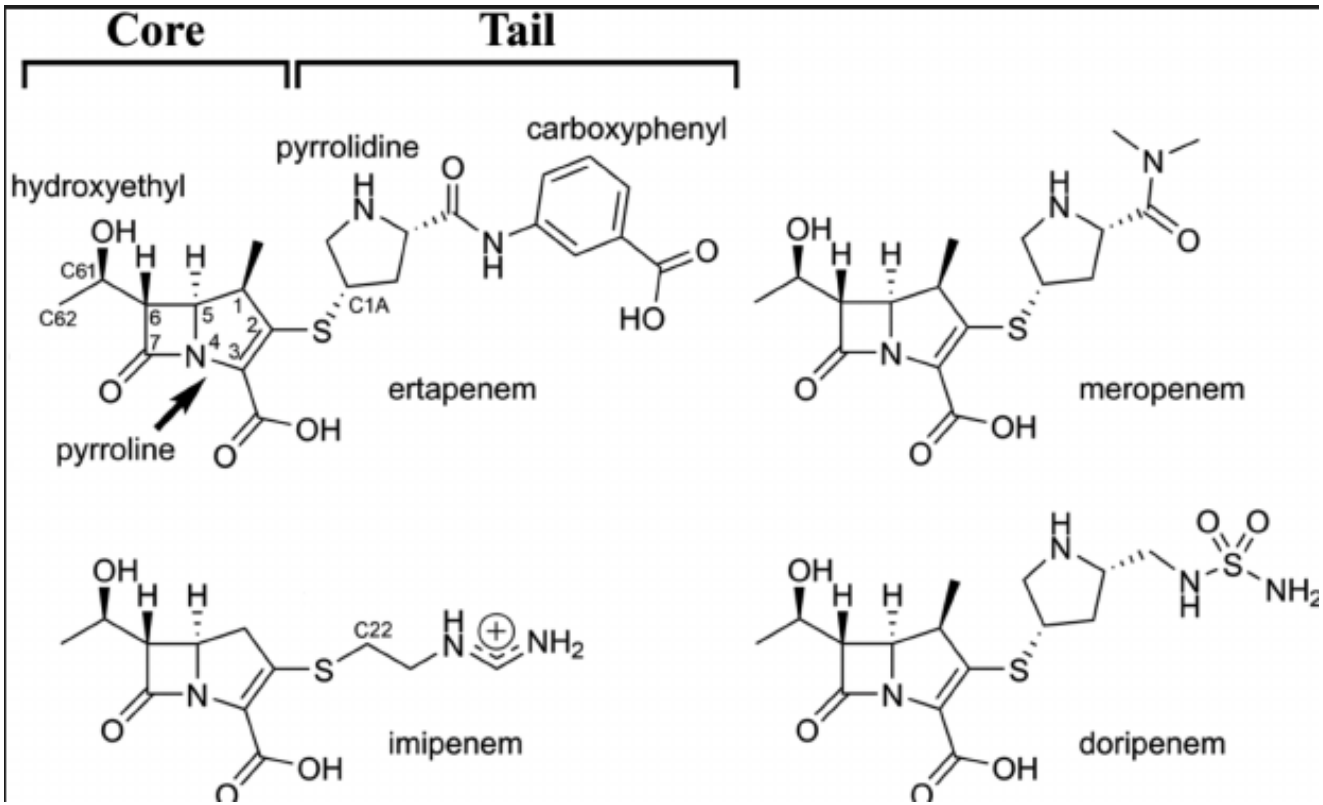
AAC 1999;43(2):424-7 NEJM 2008;358:1271-81

CRE vs CPE (CP-CRE)

Carbapenem resistant Enterobactericeae

เชื้อดื้อยาระดับเทพ

Carbapenem = Broad spectrum antibiotic = ยาฆ่าเชื้อระดับเทพ



CRE vs CPE (CP-CRE)

- **Definition**

- **CRE** = Carbapenem Resistance Enterobacteriaceae

CDC 2015 definition

Resistance to imipenem, meropenem, doripenem or ertapenem OR documentation that the isolate produce carbapenemase

- **CP-CRE** = Carbapenem-Producing Enterobacteriaceae

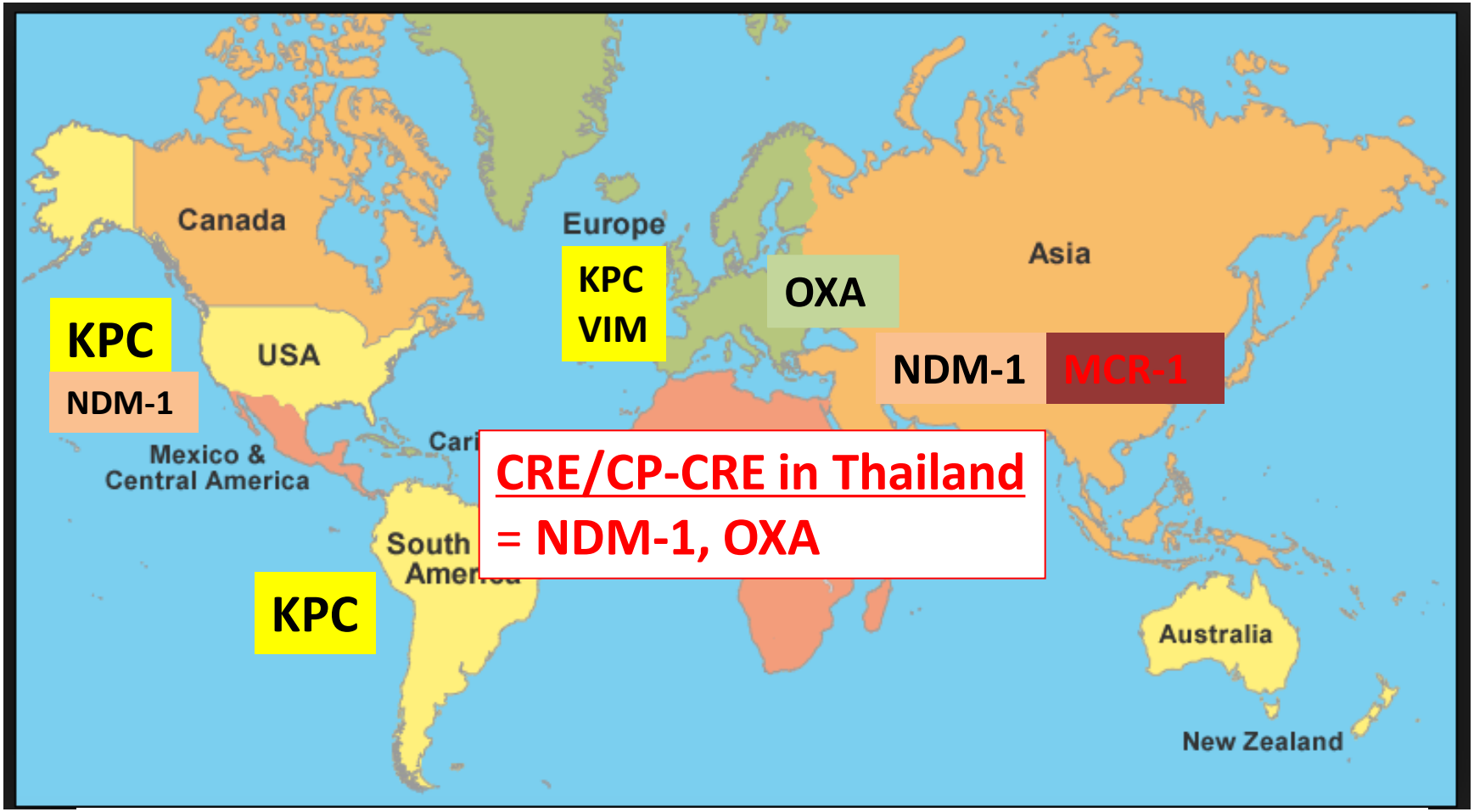
→ plasmid transferable gene (carbapenemase)

→ → Infection control implementation needed

CDC; Healthcare associate infection

CRE vs CPE (CP-CRE)

Carbapenem resistant Enterobactericeae



Variable in geographic distribution (Genotypic resistance)