NDM-1的診斷、治療與醫院感染管制

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課程大綱

1. NDM-1 MBL簡介

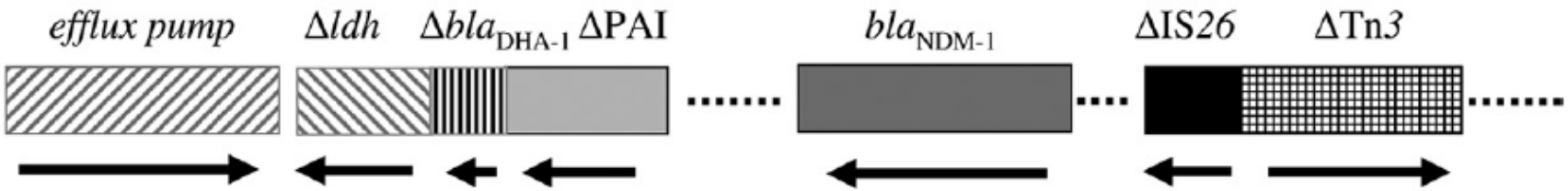
2. NDM-1 MBL的偵測與治療

3. 具有NDM-1 MBL腸道菌的預防與管制

1. NDM-1 MBL簡介

何謂NDM-1 MBL

- NDM-1 MBL
  - New-Delhi metallo β-lactamase.
  - 一段位於plasmid上的抗藥性基因。
  - 最早發現於2008年一名曾在印度半島住院的印裔瑞典人，身上所分離出來的*K. pneumoniae*。



What Is NDM-1 MBL?

- Carbapenemase
  - 顧名思義，可以水解carbapenem類抗生素的水解酶。
  - 常見的包含：
    - Metallo-β-lactamase (MBL), expanded-spectrum oxacillinase (OXA), and clavulanic-acid-inhibited β-lactamase.
    - 其中，MBL不同於它類在於活化中心為鋅離子。
  - MBL可以水解所有的β-lactam類抗生素，如penicillins，cephalosporins，及carbapenems；但不能水解aztreonam與其它類抗生素：可被EDTA中和。

Ambler Classification of β-Lactamases(1/2)

Class	*Active Site*	*Enzyme Type*	*Substrates*
A	Serine	**Penicillinases**	
		Broad-spectrum	Benzylpenicillin, aminopenicillins, carboxypenicillins, ureidopenicillins, narrow-spectrum cephalosporins
		Extended-spectrum (ESBL)	Substrates of broad-spectrum plus oxymino-β-lactams (cefotaxime, ceftazidime, ceftriaxone) and aztreonam
		Carbapenemases	Substrates of extended-spectrum plus cephamycins and carbapenems
B	Metallo-β-lactamases (Zn^{2+})	**Carbapenemases**	Substrates of extended-spectrum plus cephamycins and carbapenems
C	Serine	**Cephalosporinases**	Substrates of extended-spectrum plus cephamycins
D	Serine	**Oxacillinases**	
		Broad-spectrum	Aminopenicillins, ureidopenicillins, cloxacillin, methicillin, oxacillin, and some narrow-spectrum cephalosporins
		Extended-spectrum	Substrates of broad-spectrum plus oxymino-β-lactams and monobactam
		Carbapenemases	Substrates of extended-spectrum plus cephamycins and carbapenems

資料來源：Mandell, 7th Edition

Ambler Classification of β-Lactamases(2/2)

Class	Active Site	Enzyme Type	Examples
A	Serine	**Penicillinases**	
		Broad-spectrum	PC1 in *Staphylococcus aureus* TEM-1, SHV-1 in *Escherichia coli, Klebsiella pneumoniae*, and other gram-negative bacteria
		Extended-spectrum (ESBL)	In Enterobacteriaceae: TEM-derived, SHV-derived, CTX-M–derived; PER-1, VEB-1, VEB-2, GES-1, GES-2, IBC-2 in *Pseudomonas aeruginosa*
		Carbapenemases	KPC-1, KPC-2, KPC-3 in *K. pneumoniae*; NMC/IMI, SME family
B	Metallo-β-lactamases (Zn^{2+})	**Carbapenemases**	IMP, VIM, GIM, SPM, SIM lineages in *P. aeruginosa*, *Acinetobacter* spp. NDM-1
C	Serine	**Cephalosporinases**	AmpC-type enzymes in Enterobacteriaceae, *Acinetobacter baumannii*
D	Serine	**Oxacillinases**	
		Broad-spectrum	OXA-family in *P. aeruginosa*
		Extended-spectrum	OXA-derived in *P. aeruginosa*
		Carbapenemases	OXA-derived in *Acinetobacter* spp.

資料來源：Mandell, 7th Edition

Carbapenemase的重要性

- 對於革蘭氏陰性菌來說，carbapenem抗生素扮演了終極對抗的角色。
- 一旦細菌產生了carbapenemases，因而對carbapenems產生抗藥（此時通常也伴隨著對其它類抗生素的抗藥性），將使其治療陷入困境。

NDM-1 MBL為何受到重視？

- 高度抗藥性
  - 目前已發現能分泌NDM-1 MBL的腸道菌，除了對β-lactam類抗生素抗藥外，都帶有其他的抗藥性機轉，以致於同時對許多其它類的抗生素均有抗藥性。
- 位於plasmid上，可以在不同細菌間傳遞。
  - 容易導致群突發。
  - 以往十分受到重視的是KPC enzyme。
  - NDM-1為後起之秀。

分泌NDM-1 MBL腸道菌的流行病學

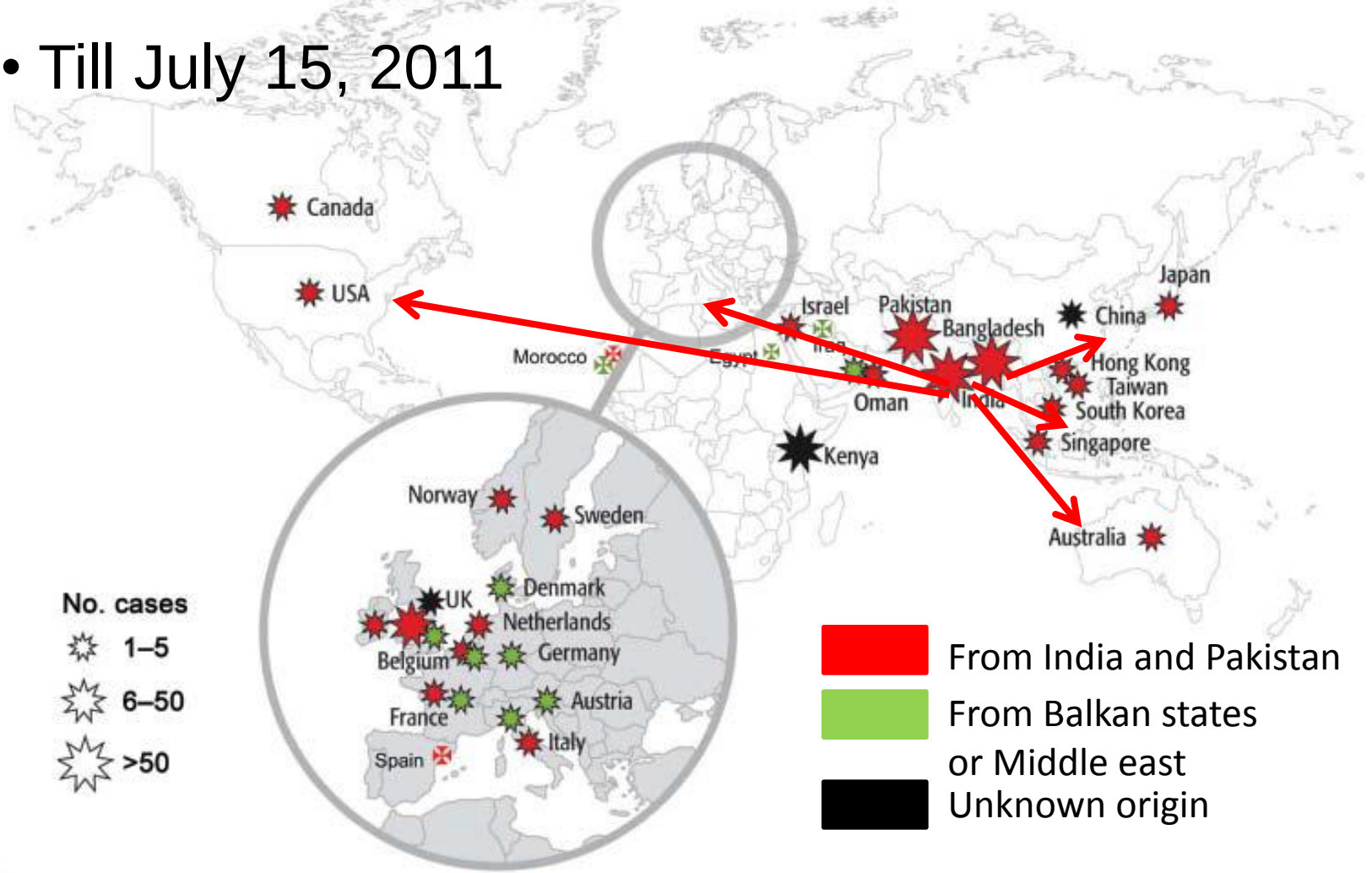
- 截至2010年8月份的資料顯示，分離出NDM-1腸道菌的個案大多發生於印度半島。
- 在美國、加拿大、荷蘭、澳洲、瑞典、法國、日本、香港、中國大陸、非洲等地，也陸續有案例報告。
  - 日本爆發院內群突發事件。
  - 英國、澳洲也發出案例增加的警訊。
  - 在印度半島以外地區發現的個案仍以曾有在印度半島接受侵入性治療和手術等就醫史為主。

Epidemiology of NDM-1 MBL

資料來源：Rolain JM, et al. Clin Microbiol Infect 2010; 16:1699-1701

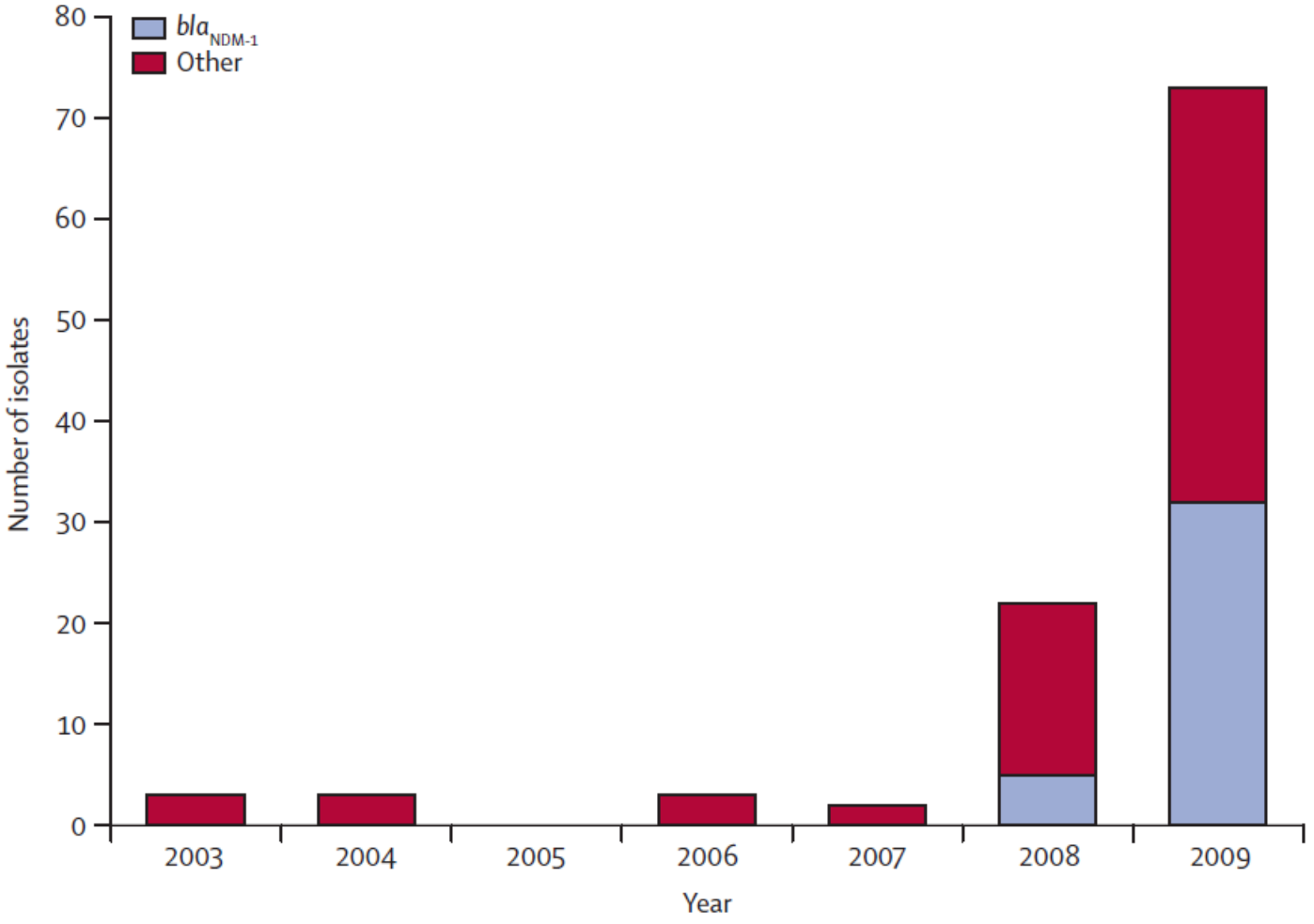
The NDM-1 → Novel Phenotype

- Till July 15, 2011



資料來源：Emerg Infect Dis. 2011 Oct; 17:1791-8

增加的趨勢：以英國實驗室為例



資料來源： Kumarasamy KK, et al. Lancet Infect Dis. 2010; 10:597-602

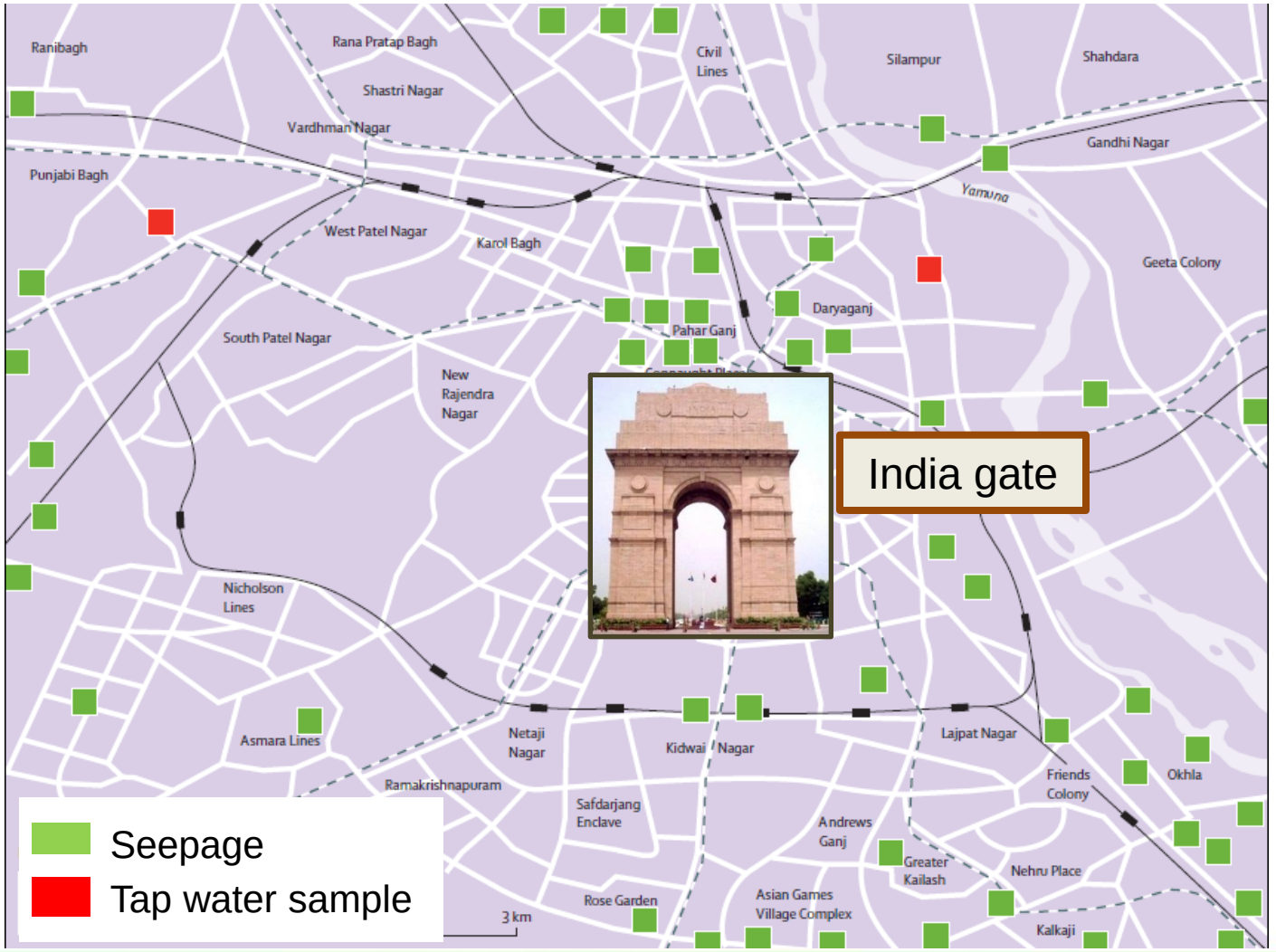
Dissemination of NDM-1 Positive Bacteria in the New Delhi Environment

- In tap and environment water in New Delhi.

	Seepage water N=171	Tap water N=50
bla$_{NDM-1}$ (+)	51/171	2/50

- Disseminate among species.
  - Not only Enterobacteriaceae.
  - But also *Aeromonads*, *Vibrio*, and various non-fermenters.

The NDM-1



資料來源： Lancet Infect Dis 2011; 11: 355–62

新聞案例一

- 食尚玩家印度中彈攝影師染超級細菌
  - First identification of patient colonized with *K. pneumoniae* carrying bla_{NDM-1} in Taiwan.
- NDM-1 appearance
  - Returned from New Delhi where he had been hospitalized for a gun-shot injury status post operation.
  - *K. pneumoniae* isolates carrying bla_{NDM-1} were isolated from 2 consecutive fecal samples from anal swabs.

資料來源：聯合報
J Chin Med Assoc 2010; 73:596–98

新聞案例二(1/2)

超級細菌NDM-1 本土首感染

但誰也沒想到，事隔3個多月，疾病管制署突然召開記者會，表示發現了第一例本土超級細菌NDM-1感染病例。

這名男子，2010年10月在中國動手術回台後，因為下腹疼痛就醫，醫師發現他白血球下降，腹腔積水，在11/17抽取的腹腔引流液，11/27採取的尿液，還有12/21的尿液中，都培養出抗藥性克雷白氏菌，高度懷疑就是NDM-1惹的禍，1/10送細菌株到疾病管制署，1/13疾病管制署確認就是NDM-1。

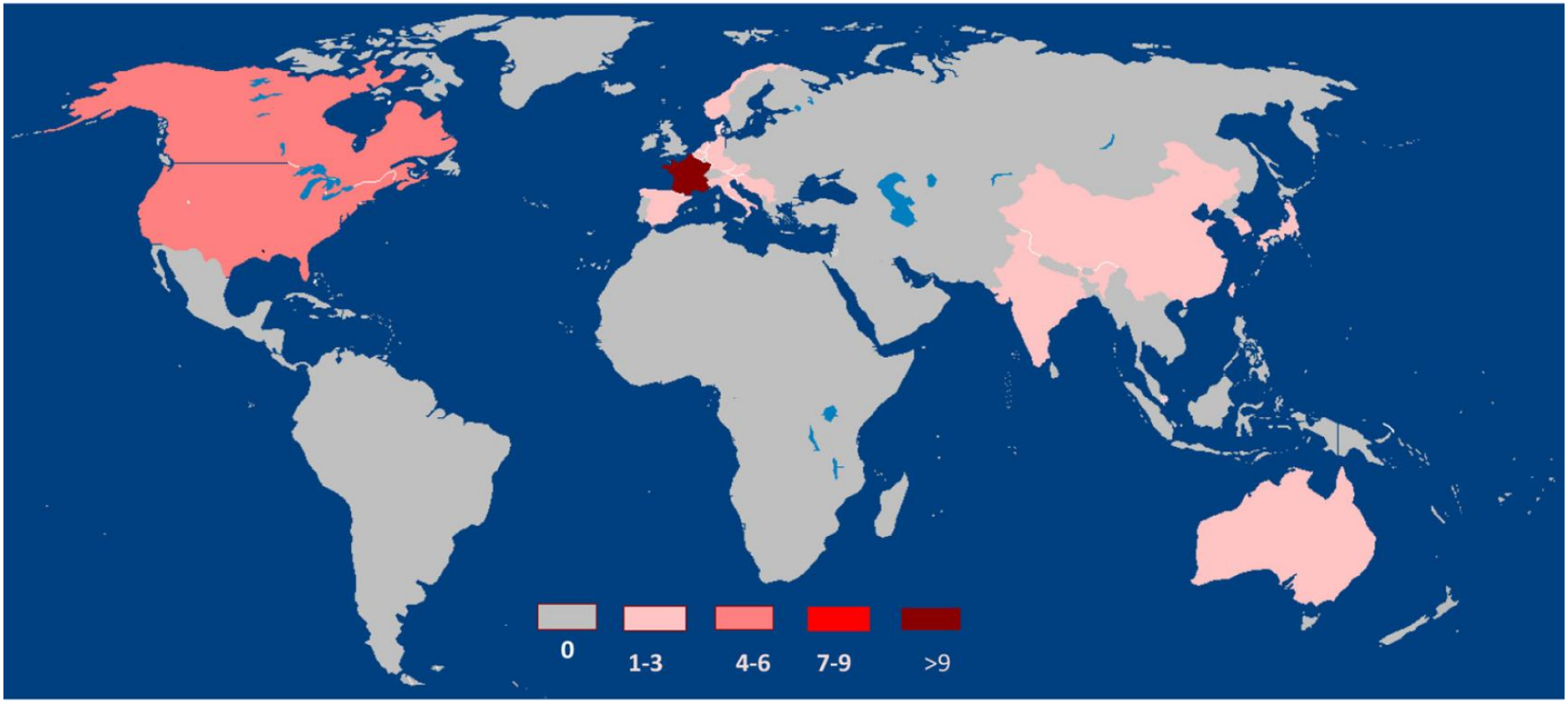
資料來源：民視新聞
Diagn Microbiol Infect Dis. 2011 Dec; 71:474-5

新聞案例二(2/2)

- Pelvic abscess caused by New Delhi metallo-β-lactamase-1-producing *Klebsiella oxytoca* in Taiwan in a patient who underwent renal transplantation in China.
- NDM-1 appearance
  - 4 isolates(2 from pigtail drainage fluid on 16th and 27th day; 2 from foley catheter on 38th and 62th) yielded *K. oxytoca* carrying $bla_{\text{NDM-1}}$.
  - Neither anal nor throat swab cultures yielded ertapenem-resistant isolates.

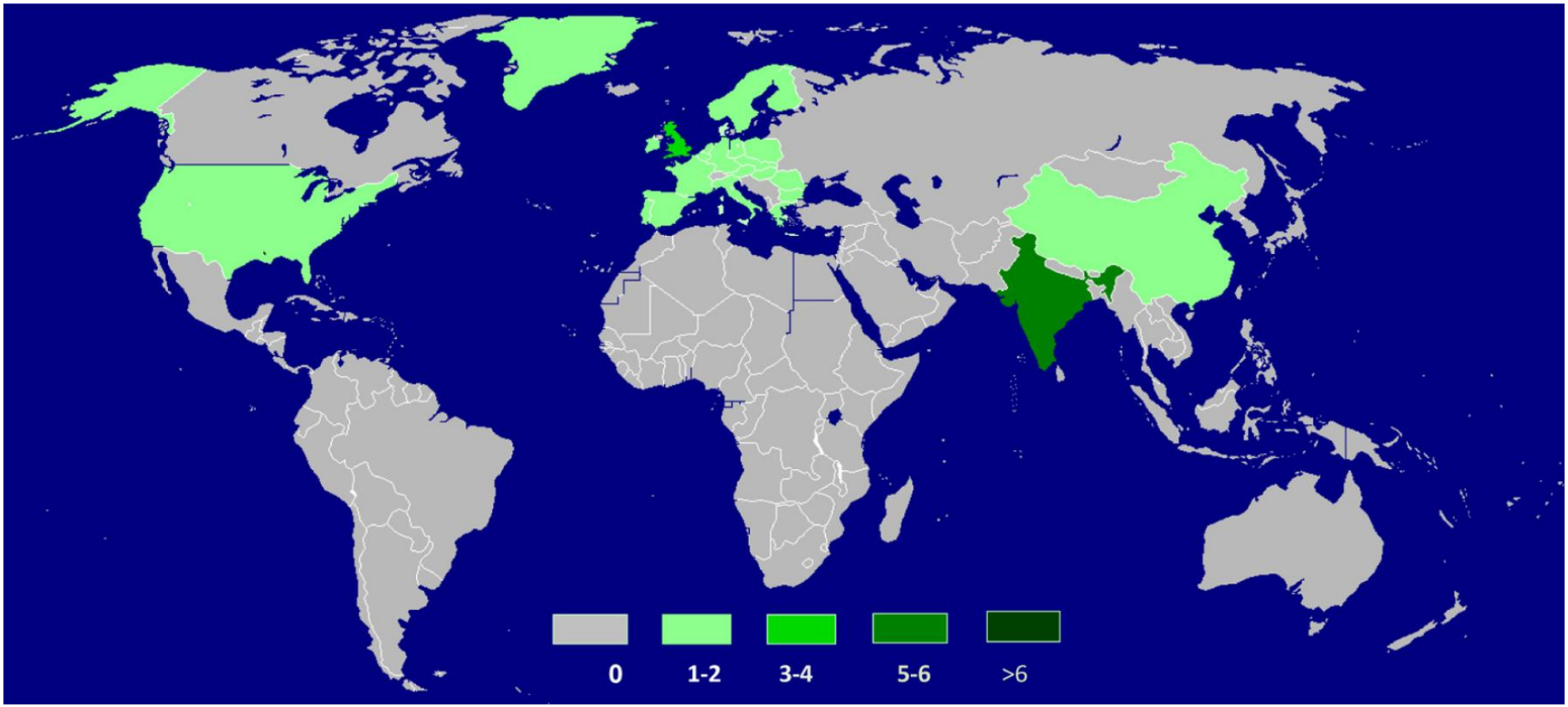
資料來源：Diagn Microbiol Infect Dis. 2011 Dec; 71:474-5

Burden of NDM-Producing Enterobacteriaceae



資料來源：Bushnell G, et al. Int J Infect Dis 2013; 17:e325-333

Burden of NDM-Producing Non-Enterobacteriaceae



資料來源：Bushnell G, et al. Int J Infect Dis 2013; 17:e325-333

哪些腸道菌曾被發現帶有NDM-1 MBL

Bacteria	Infection source (n=25)				Colonization source (n=60)					Total
	Urine	Blood	Wound	Resp	Urine	Stool	Resp	Wound	Others	
K. pneumoniae	5	1	1	2	13	8	4	2	4	40
K. oxytoca	-	-	1	-	-	-	-	-	-	1
E. coli	4	3	5	-	2	9	4	1	-	28
Providencia spp.	-	1	-	-	2	-	-	-	-	3
C. freundii	1	-	-	-	-	-	-	-	-	1
M. morganii	-	-	-	-	1	1	-	1	-	3
E. cloacae	-	-	-	-	1	-	-	1	-	2
P. mirabilis	-	-	-	-	-	1	-	-	-	1
A. baumannii	-	1	-	-	-	-	1	1	1	4
P. aeruginosa	-	-	-	-	1	-	-	1	-	2
Total	10	6	6	2	20	19	9	7	5	84

資料來源：Bushnell G, et al. Int J Infect Dis 2013; 17:e325 – 333
Lai CC, et al. Diagn Microbiol Infect Dis. 2011 Dec; 71:474-5

2. NDM-1 MBL的偵測與治療

如何偵測NDM-1?

- Screening
  - Carbapenem resistance
- Confirmatory tests
  - Modified Hodge test
  - Combined disk/disk synergy method
  - UV spectrophotometer, Mass spectrometry, Carba NP test
  - MBL Etest
  - PCR detection: gold standard

Definitions of CRE by US CDC

Nonsusceptible to one of		Resistant to all of
• Doripenem • Meropenem • Imipenem	And	• Ceftriaxone • Cefotaxime • Ceftazidime

➡ In Taiwan, no consensus at current time.

MIC Breakpoint of Enterobacteriaceae to Carbapenem

- US(CLSI) V.S. European(EUCAST)

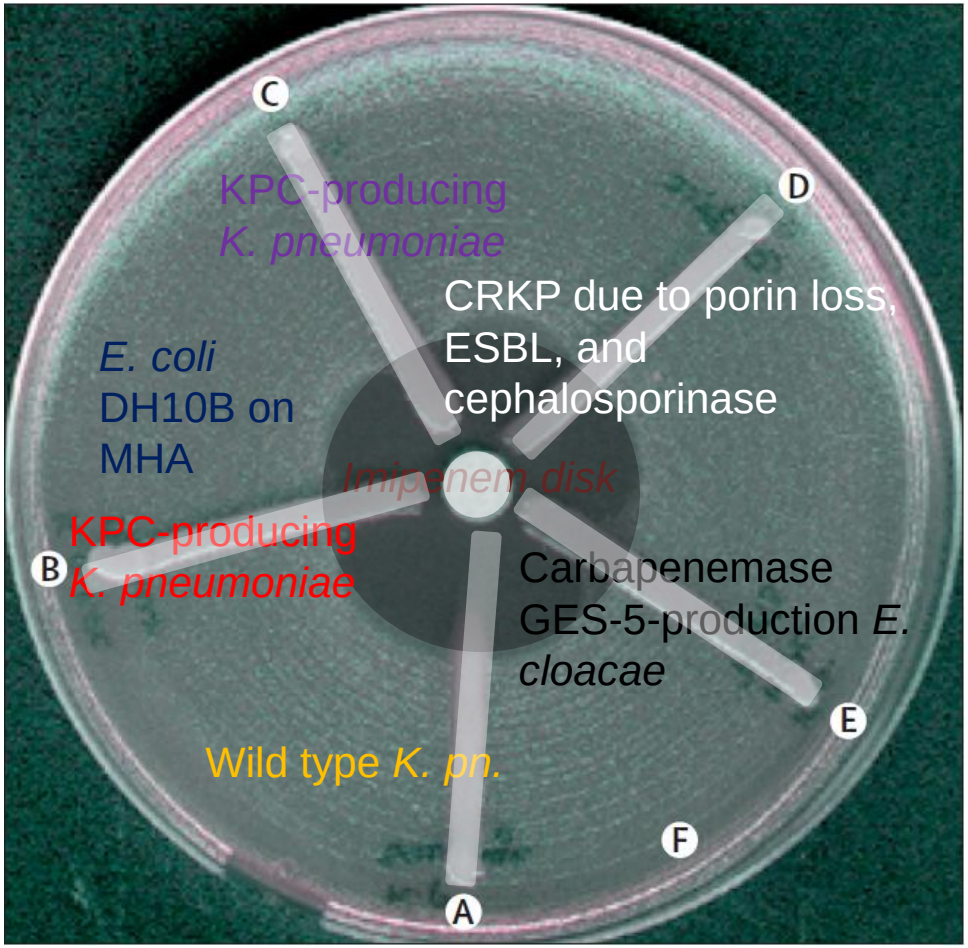
CLSI	Previous Breakpoint MIC (ug/ml)			Current Breakpoint MIC (ug/ml)		
	S	I	R	S	I	R
Doripenem	-	-	-	≤1	2	≥4
Ertapenem	≤2	4	≥8	≤0.5	1	≥2
Imipenem	≤4	8	≥16	≤1	2	≥4
Meropenem	≤4	8	≥16	≤1	2	≥4

EUCAST	S (≤)	R (>)
Doripenem	2	8
Ertapenem	0.5	1
Imipenem	2	8
Meropenem	2	8

Update in 2010

資料來源：Clin Microbiol Infect. 2012 May; 18:432-8
CLSI document M100-S22. Wayne, Pennsylvania, 2012

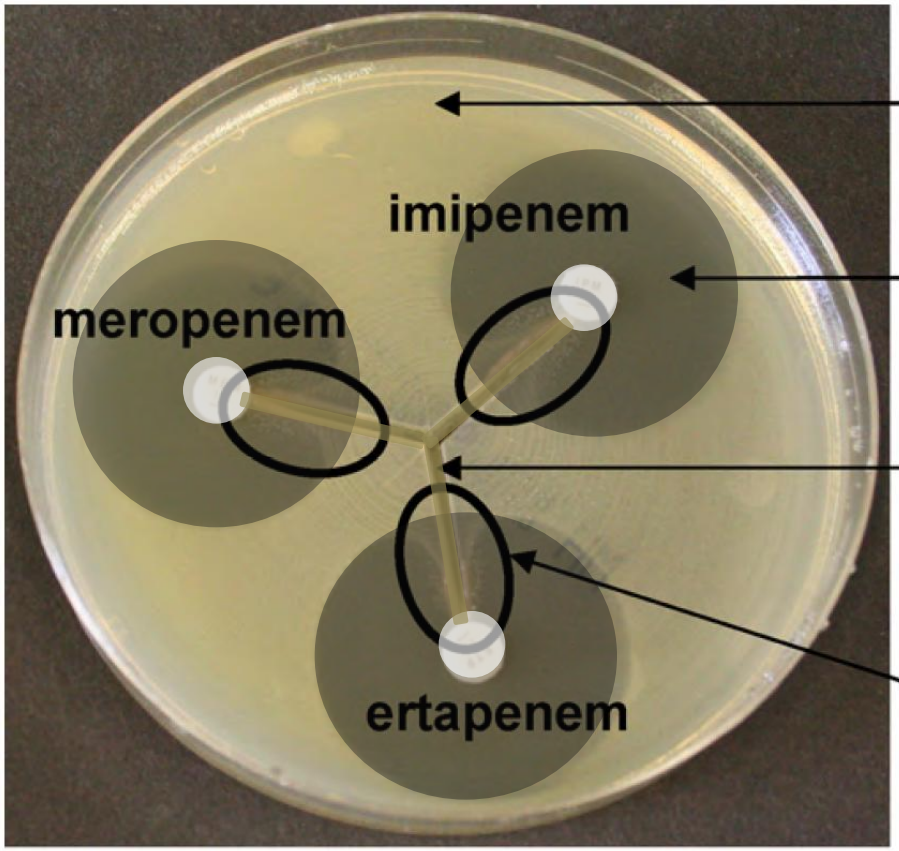
The Modified Hodge Test(1/2)



- The problem is that this detect. carbapenemases, not exclusive for NDM-1.

資料來源：http://image.thelance.com

The Modified Hodge Test(2/2)



Growth of *E. coli* ATCC 25922

Inhibition of *E. coli* ATCC 25922

Test isolate (suspected carbapenemase production)

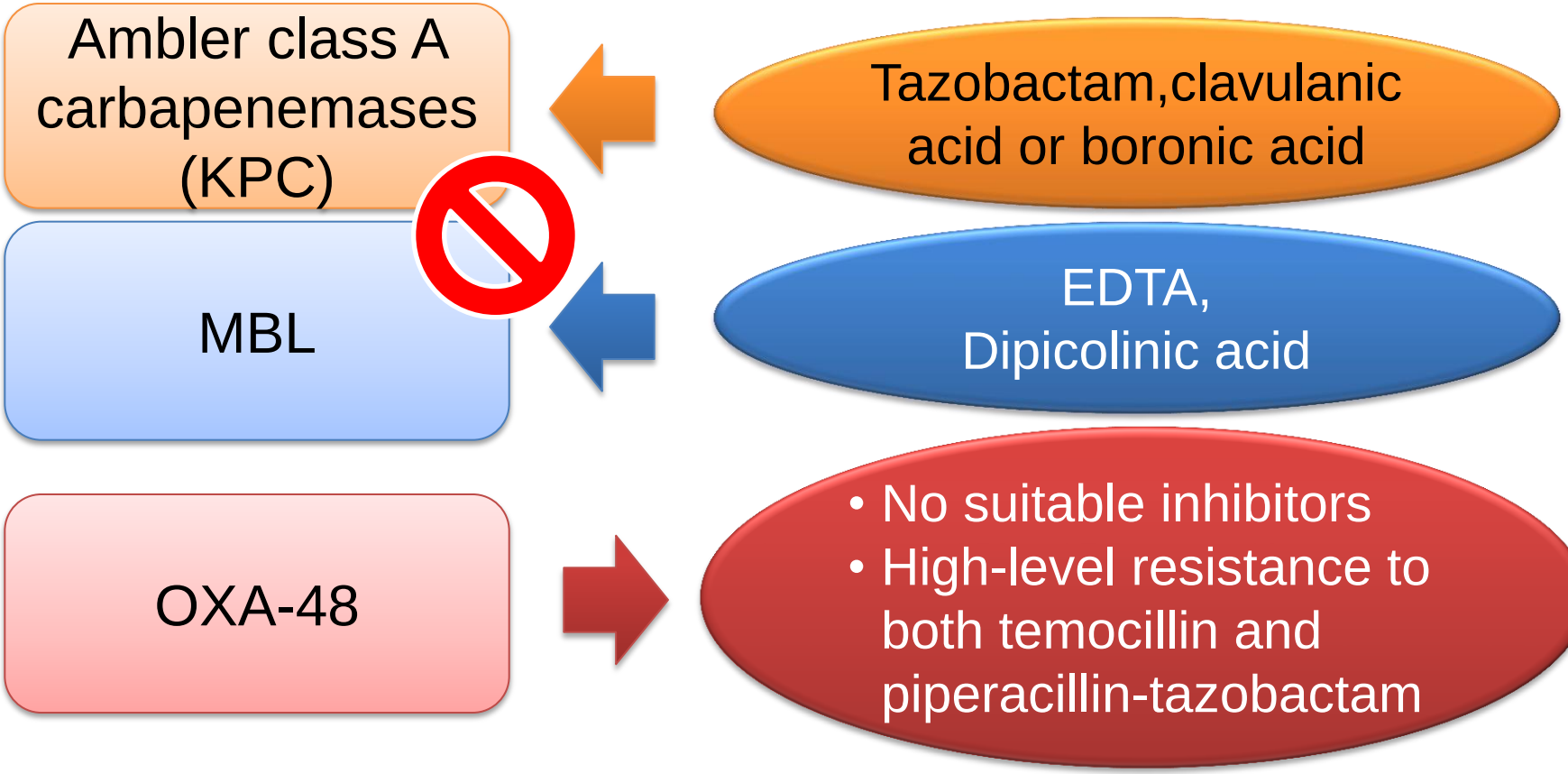
10 μg disc, 20 – 25 mm long

Increased growth of *E. coli* ATCC 25922 (disturbed edge of inhibition zone) by inactivation of the carbapenem from the disk by diffusion of the carbapenemase originating from the suspected isolate in the streak

資料來源：Stuart JC, et al. Int J Antimicrob Agents 2010; 36:205–10

Disk Synergy Test or Combined Disk Test(1/3)

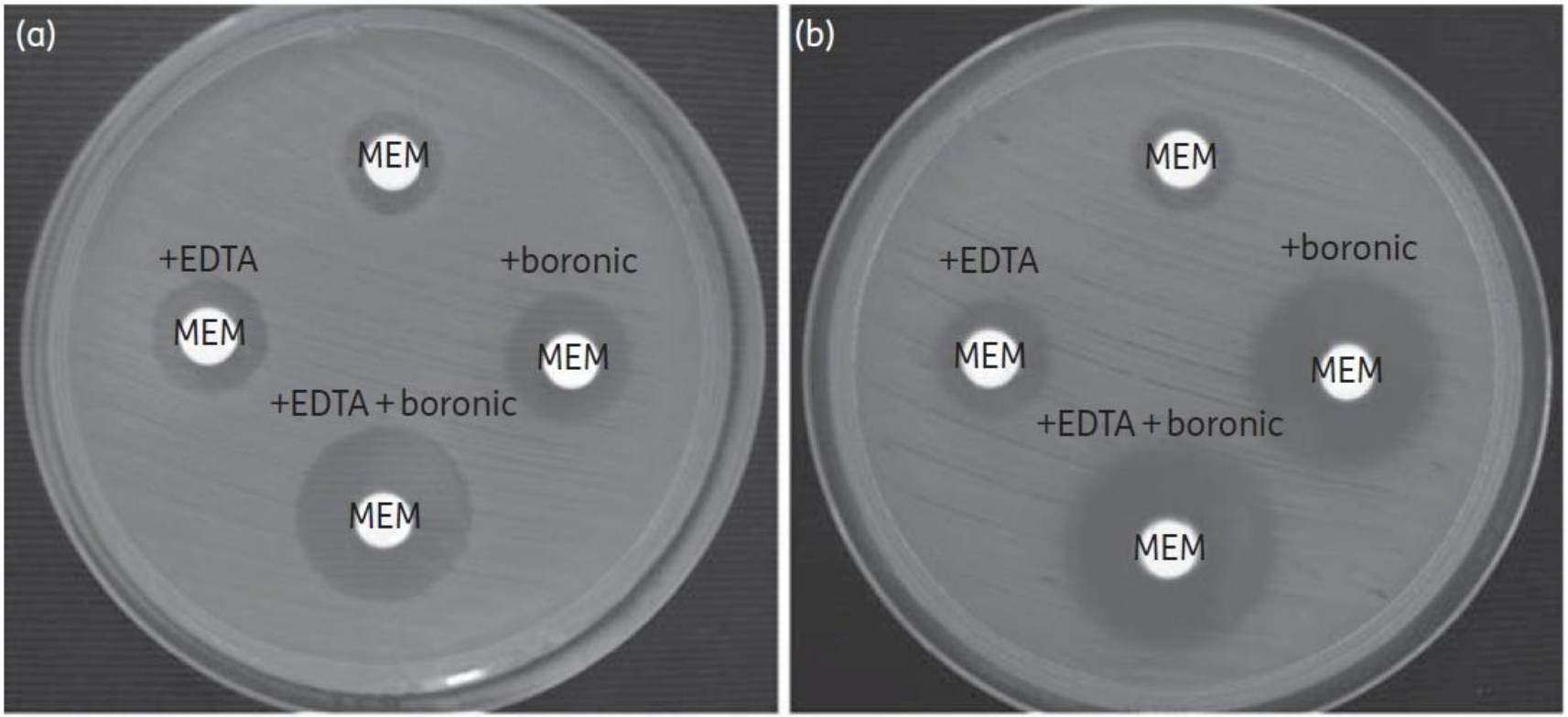
- Inhibition-based carbapenemase detection



資料來源：Indian J Med Microbiol. 2011 Jul-Sep; 29:275-9
J Antimicrob Chemother. 2013 Mar; 68:487-9

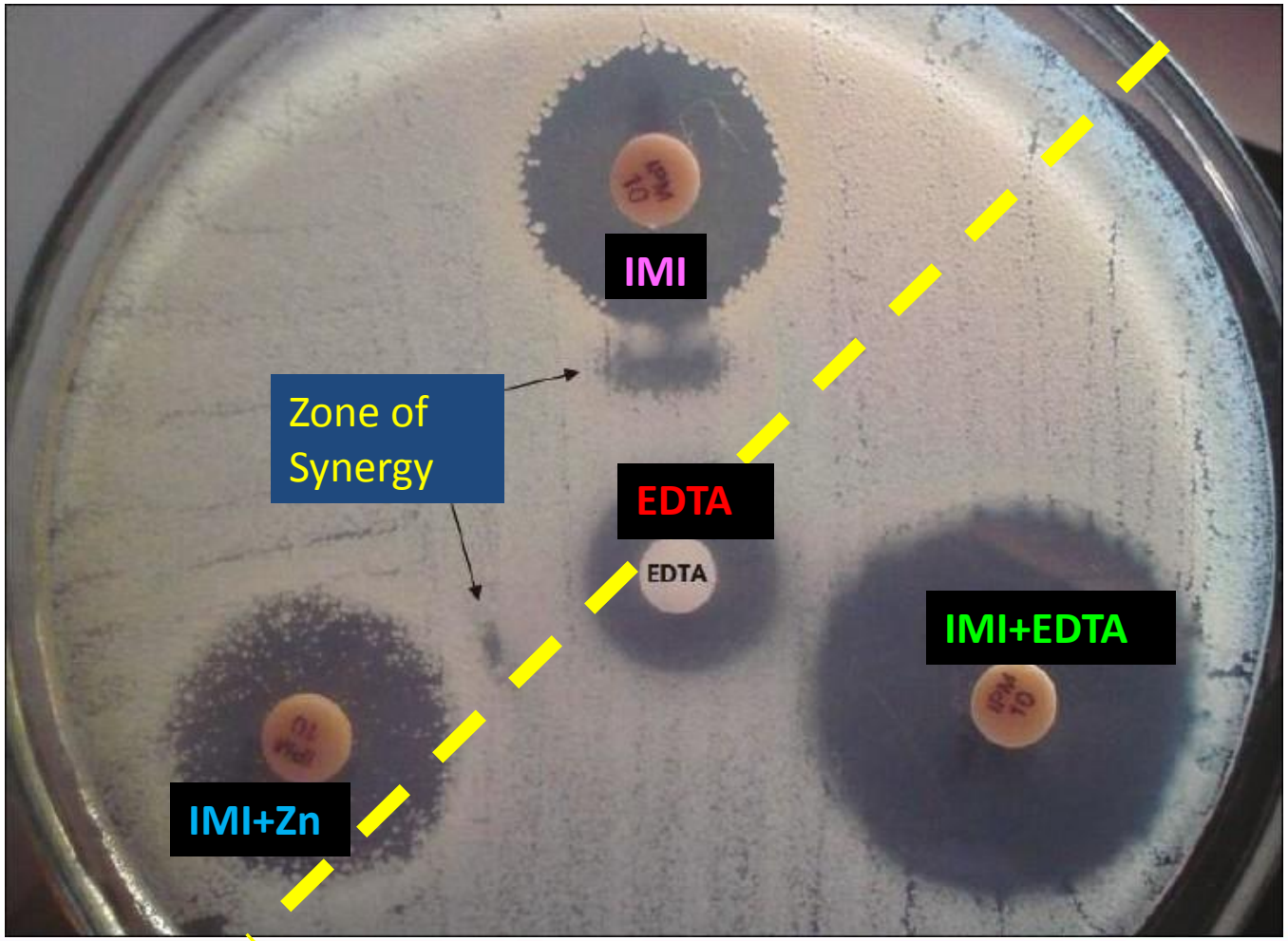
Disk Synergy Test or Combined Disk Test(2/3)

- Examples of combined disk test



資料來源：Tsakris A, et al. J Antimicrob Chemother 2010; 65:1664–71

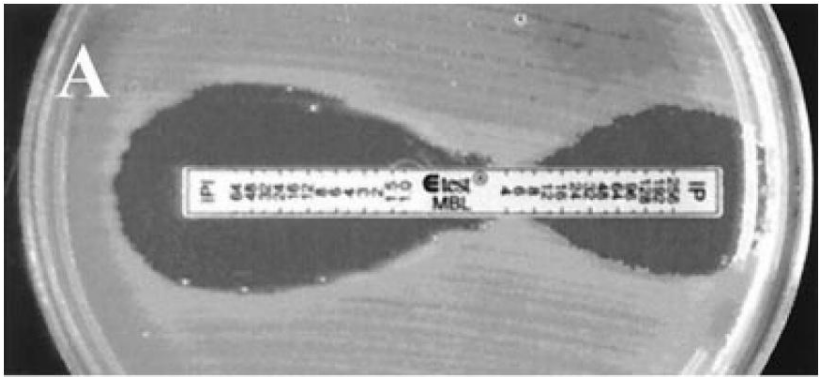
Disk Synergy Test or Combined Disk Test(3/3)

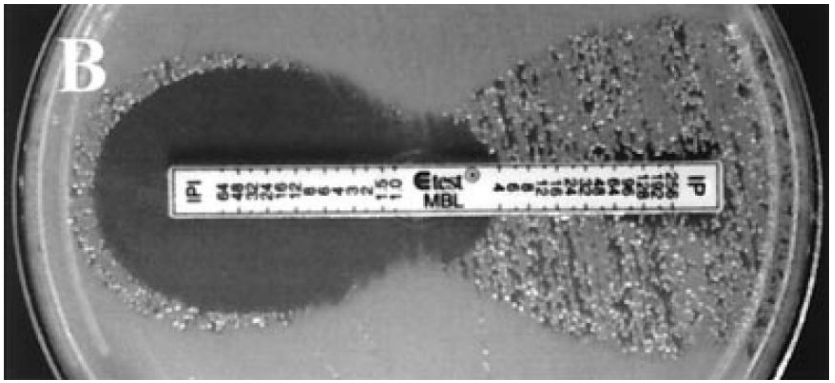


資料來源：Indian Journal of Medical Mycology; 2011; 29:275-9

MBL Etest

- Imipenem v.s. imipenem + EDTA
  - MIC ratio ≥8
  - The presence of a phantom zone





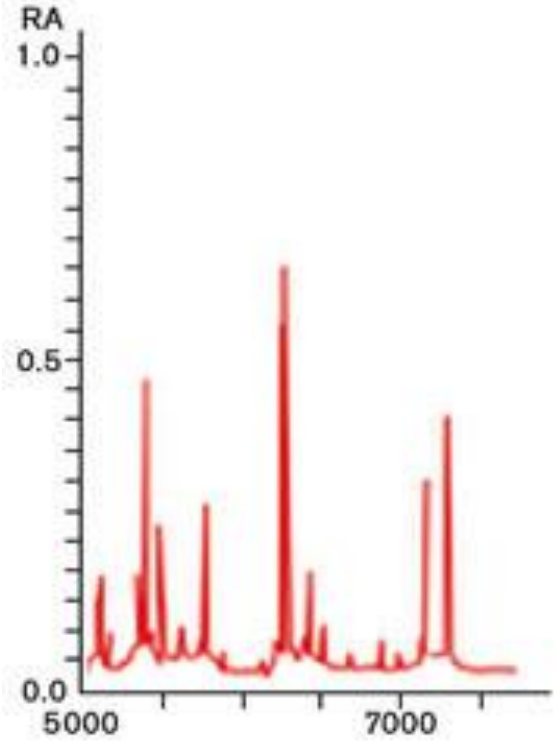
資料來源：Lee K, et al. J Clin Microbiol 2005; 43:942–44

UV Spectrophotometer

- Measurement of imipenem hydrolysis (wavelength: 297nm)
- Couldn’t discriminate the different types of carbapenemase

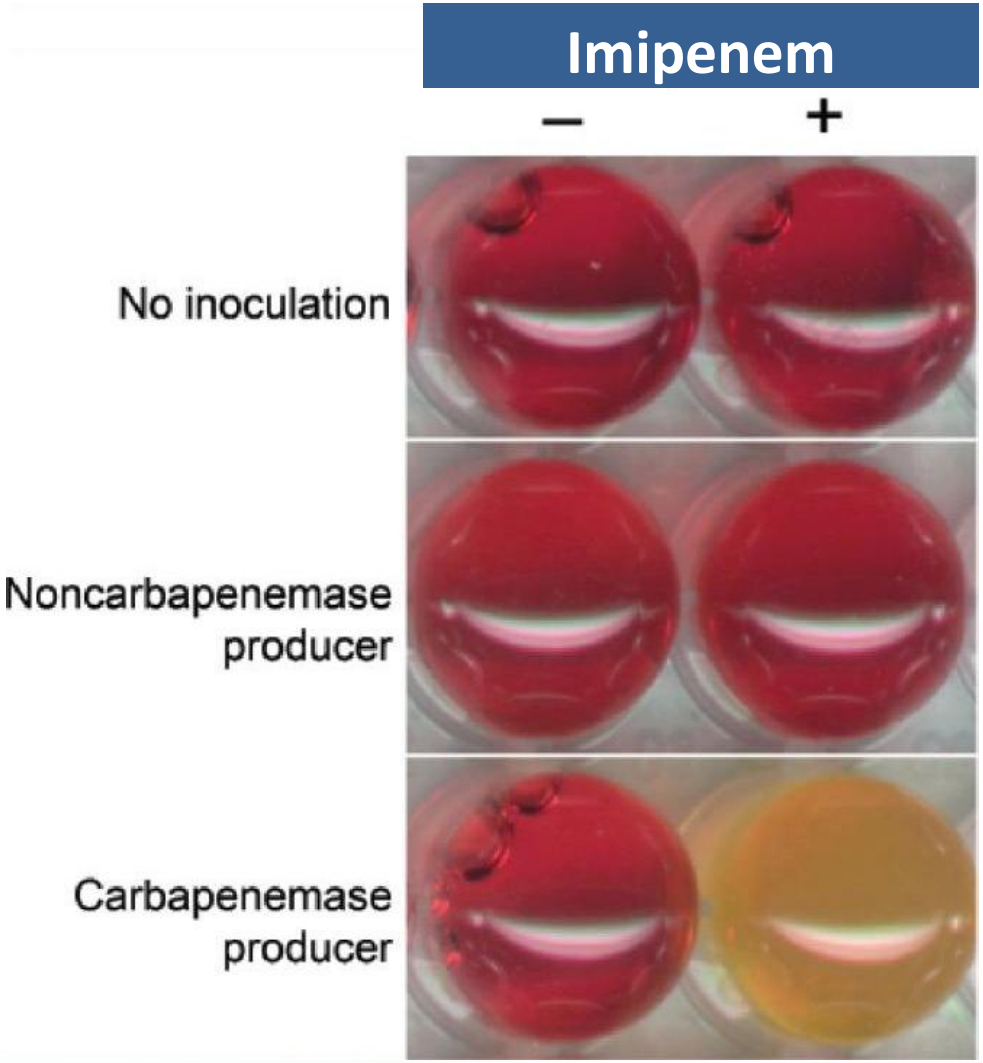
Mass Spectrometry

- Analysis of the degradation of a carbapenem molecule



資料來源：J Antimicrob Chemother. 2013 Mar; 68:487-9

Carba NP Test



- Hydrolysis of imipenem is detected by a change in the pH value of the indicator
- Identify virtually any new emerging carbapenemase
- Rapid (2 h), user-friendly and inexpensive technique

資料來源：J Antimicrob Chemother. 2013 Mar; 68:487-9

Confirmation of NDM-1

- PCR detection
  - Primers 1

NDM-1-F	• ATGCGGGCCGTATGAGTGA
NDM-1-R	• GGAAACTGGCGACCAACG

  - Primers 2

NDM-2-F	• CTCATGTTTGAATTCGCCC
NDM-2-R	• ACTCGTCGCAAAGCCCAG

資料來源：Limbago B, et al. Poster C1-675d, 50th ICCAAC, Boston 2010

Detection of Carbapenemase(1/2)

- Confirmatory test
  - Non-molecular-based tests
  - Molecular test: gold standard
    - Based on PCR, followed by sequencing type
    - Can give results within 4-6 hours (or less when RT-PCR)
    - Disadvantages
      - Cost and requirement for trained microbiologists
      - Inability to detect novel unidentified gene

(Need reference laboratory for further genotypic confirmation)

資料來源：J Antimicrob Chemother. 2013 Mar; 68:487-9

Detection of Carbapenemase(2/2)

Carbapenemases screen:
E-coli, Klebsiella spp.,
Enterobacter spp., Salmonella spp.,
Citrobacter spp.

Expert system (+) or
MIC meropenem >= 0.5 or
MIC Imipenem >=2.0

Carbapenemases screen:
Proteus spp., Serratia spp.,
M.morganii, Providencia spp.

Expert system (+) or
MIC meropenem >= 0.5

Positive

Elevated carbapenem MIC confirmed with meropenem or imipenem **Etest**?

Yes

Genotypic confirmation

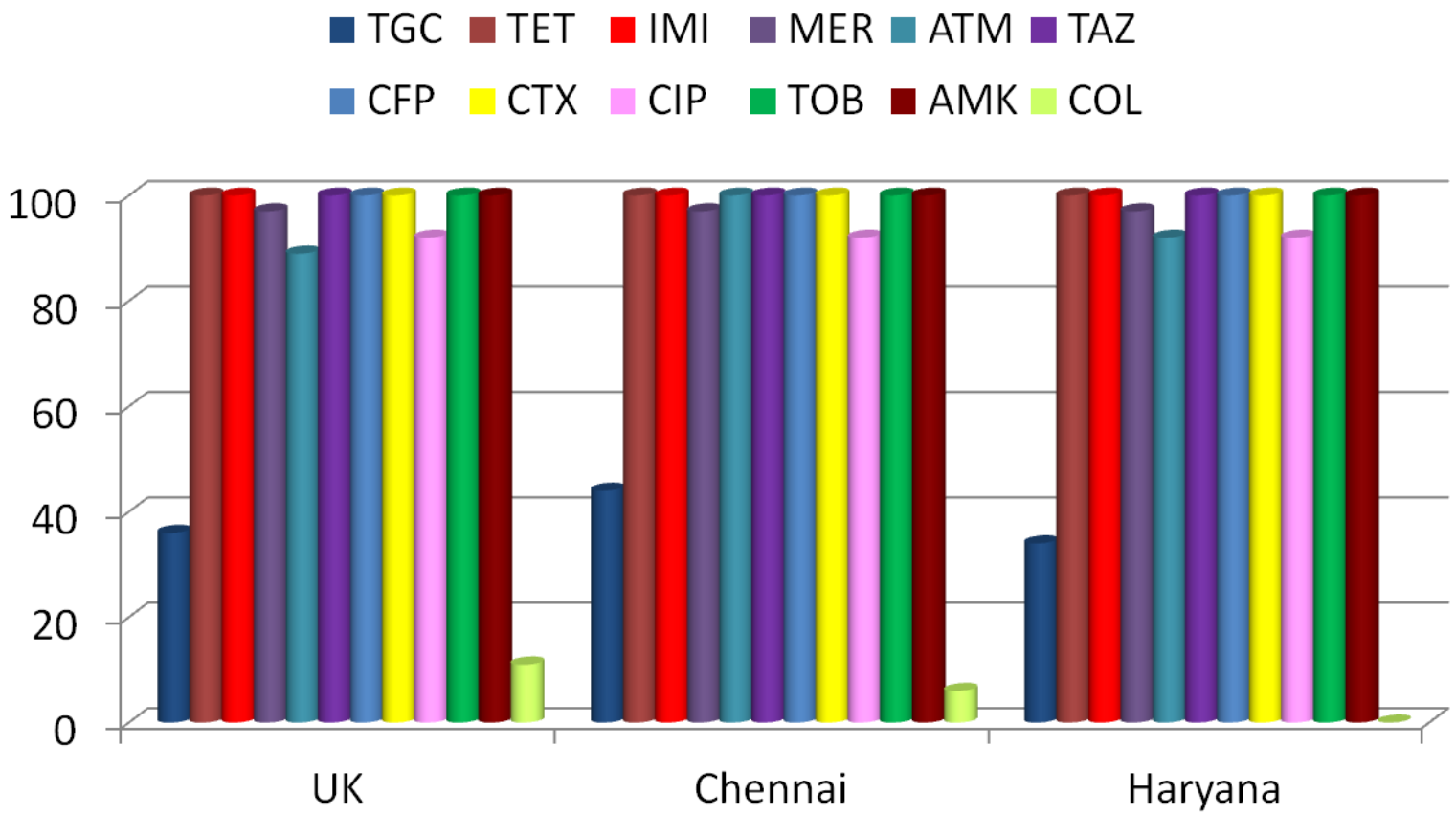
1.PCR detection
2.Sequencing of carbapenemases gene

Phenotypic confirmation

1.Modified Hodge test
2.APB-meropenem combination disc
3.Meropenem-DPA combination disc
4.Imipenem-EDTA combination disc

資料來源：Int J Antimicrob Agents. 2010 Sep; 36:205-10

治療：體外敏感性測試結果



資料來源：Kumarasamy KK, et al. Lancet Infect Dis 2010; 10:597-602

Treatment Options

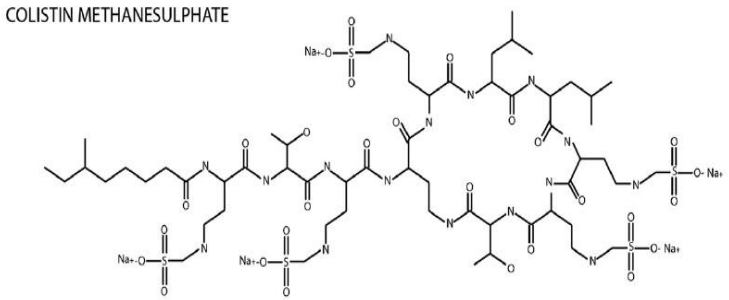
- Colistin
- Tigecycline
- Fosfomycin
- Novel Agents

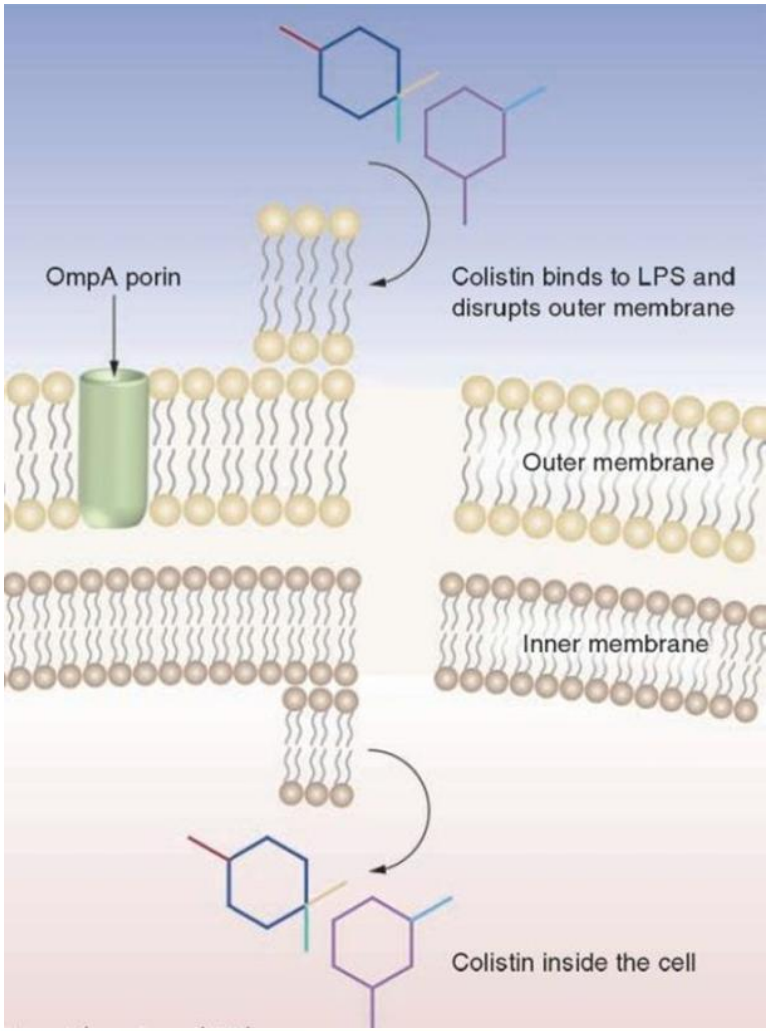
Colistin

- Polymyxin E
- FDA indication
  - *Enterobacter aerogenes*
  - *Escherichia coli*
  - *Klebsiella pneumoniae*
  - *Pseudomonas aeruginosa*

➔Not indicated

  - *Burkholderia/Proteus/ Providencia/Serratia*



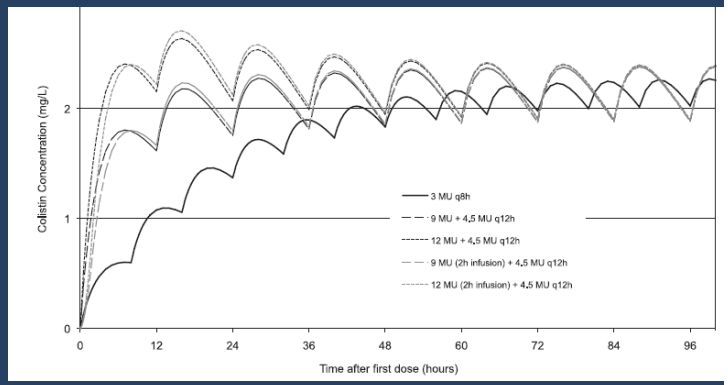


資料來源：Expert Rev Anti Infect Ther. 2012; 10:919-34

Recommended Dosage

1.Colistin sulfate (Tablet/syrup/powder)
2.Colistin methane-sulfonate (IV)

Intravenous access
Loading dose: 5mg/kg (IBW)
Maintenance dose: 2.5-5 mg/kg/day
Renal adjustment



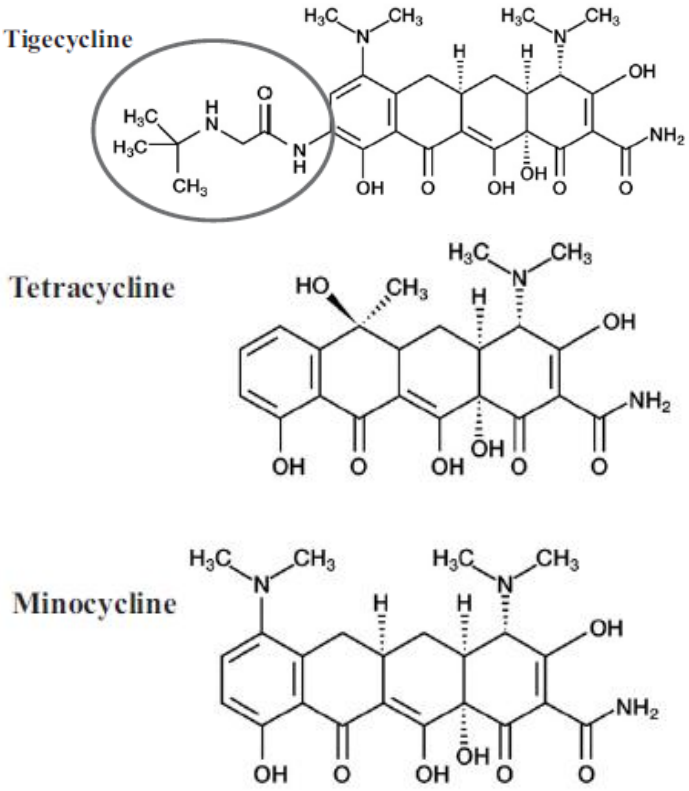
Inhalation access

Nephrotoxicity and Neurotoxicity

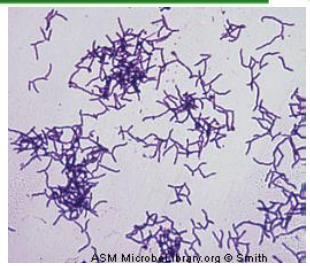
資料來源：Clin Infect Dis. 2010; 51: 1238-1244

Tigecycline

- Minocycline derivative
- Resist Tetracycline efflux pump
- FDA indication
  - cIAI: ESBL
  - cSSSI: MRSA, VRE
  - **Not indicated**
  - *Pseudomonas aeruginosa/ Proteus* spp.
  - *Providencia/Morganella morganii*
  - Low serum concentration!



Fosfomycin

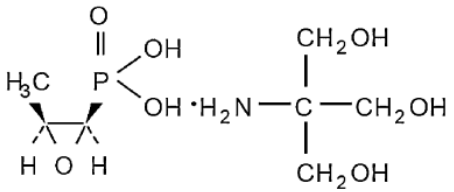


1969 Streptomyces species

Inhibit peptidoglycan synthesis
High concentration in serum and urine

Oral fosfomycin (Fosfomycin tromethamine)
UTI: *E.coli, Enterococcus faecalis*

Intravenous fosfomycin (Fosfomycin disodium)
Critical infection CRKP, MRSA, VRE



資料來源： http://www.druginformation.com/

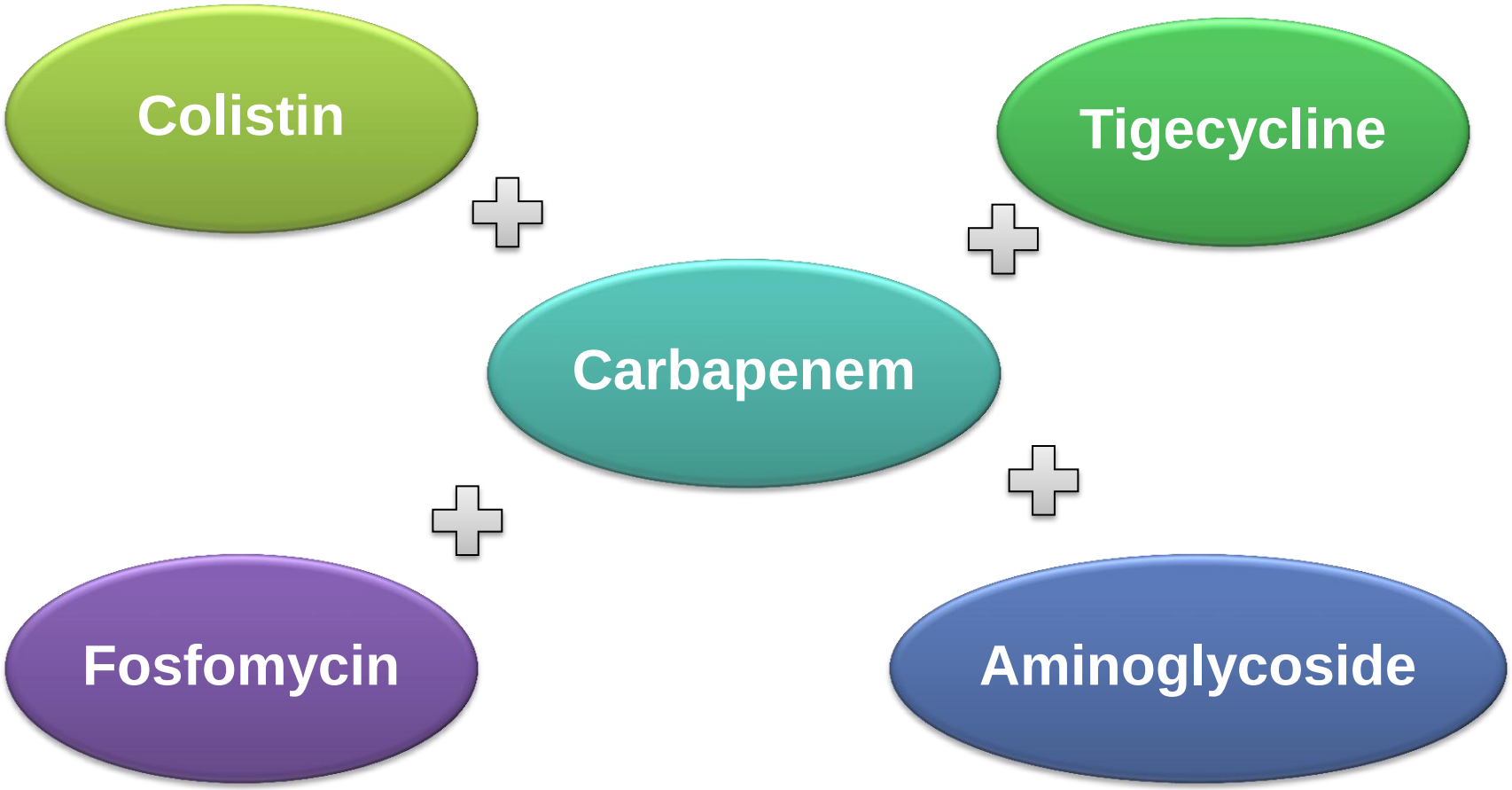
Limitation of Fosfomycin

High sodium level

Resistance develops rapidly when monotherapy!

Not available everywhere!

Combination Versus Monotherapy?

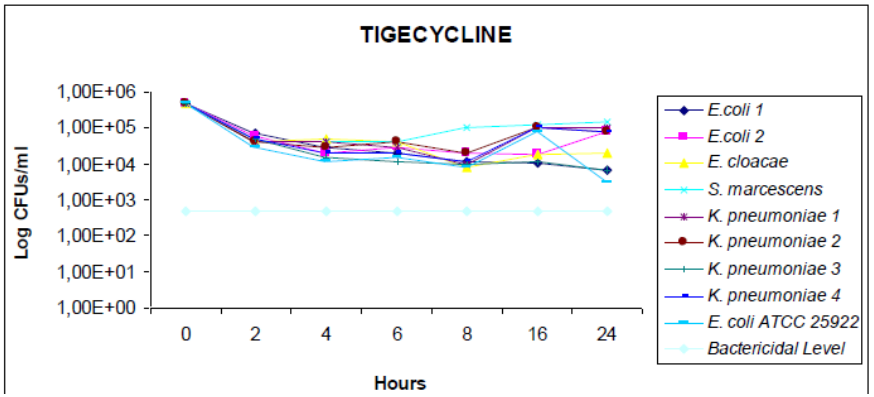


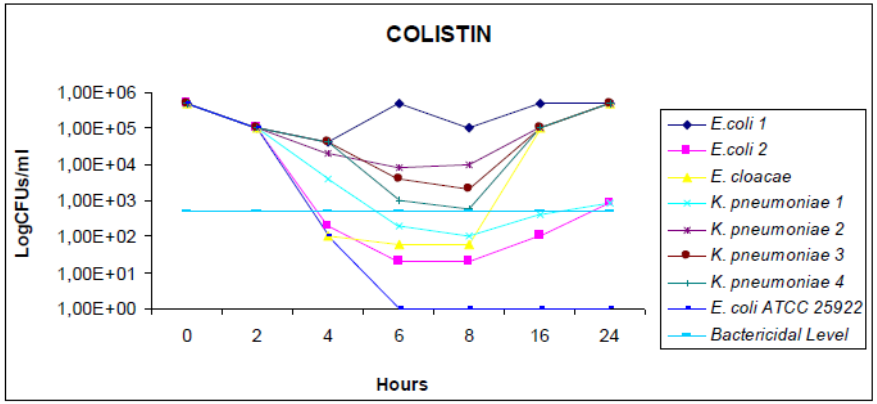
Tigecycline + Colistin

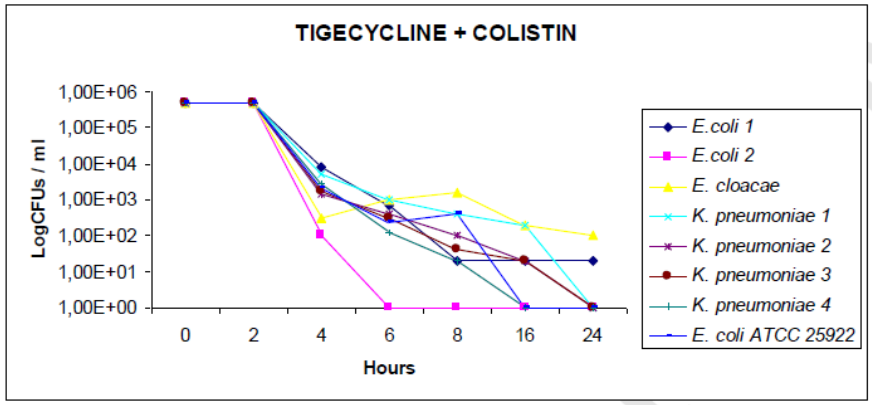
2011 Greece

8 KPC-producing Enterobacteriaceae

4 K. pneumoniae, 2 Escherichia coli, 1 Enterobacter cloacae, 1 Serratia marcescens







Synergistic effect!!

資料來源：Int J Antimicrob Agents. 2011; 37:244-7

Carbapenem + Colistin

2003/08~2004/06 Brooklyn, New York
16 isolates of Carbapenem resistant *Klebsiella pneumonia*

Antibiotic (concentration, mg/L)	No. with decrease ≥3 log cfu/mL at 24 h	
Imipenem (4)	0/16	
Polymyxin B (1)	7/16	43.7%
Polymyxin B (1) + imipenem (4)	11/16	68.7%

Synergistic effect!!

Carbapenem + Tigecycline

No decreased more than 3 log10 CFU
No synergistic effect compared with Tigecycline alone

資料來源：J Antimicrob Chemother. 2005; 56:128-32
Int J Antimicrob Agents. 2011; 37:244-7

Fosfomycin + Colistin (In Vitro)

2011 Worldwide origin
28 NDM-1 producing Enterobacteriaceae
(10 *K. pneumoniae*, 10 *Escherichia coli*, 4 *Enterobacter cloacae*, 4 Others)

Synergic in rare isolates (3 isolates)

Fosfomycin + Colistin (In Vivo)

2008/05-12 Greece
11 patients
Carbapenem-resistant *Klebsiella pneumonia*
(2 bacteremia, 3 VAP+bacteremia, 2 VAP+UTI, 2 UTI, 1 bacteremia+wound infection, 1 wound infection)

2 in 6 patients expired

資料來源：J Antimicrob Chemother. 2011; 66:2295-7
Eur J Clin Microbiol Infect Dis (2012) 31:695–701

Current Evidence?

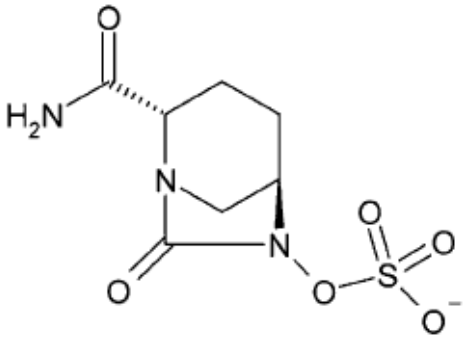
- Most studies: no significant difference in mortality between the combination and monotherapy groups.
- Three studies reporting on 194 critically ill patients with bacteremia showed individually significantly lower mortality in the combination arm.
- Clinical heterogeneity, retrospective data, a rather small number of patients.

Novel Agents

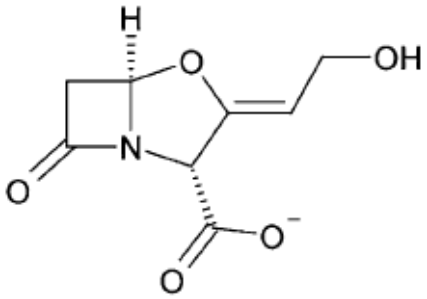
- NXL 104 (Avibactam)
- Penem-1
- BAL30376

NXL 104 (Avibactam)

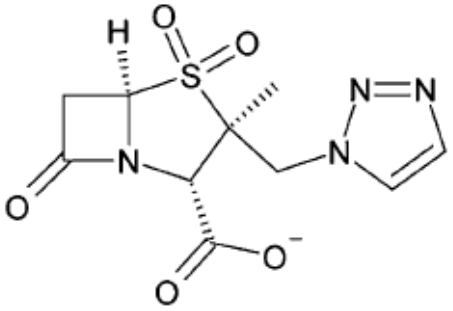
- Novel β-lactamase inhibitor



Avibactam



Clavulanic acid

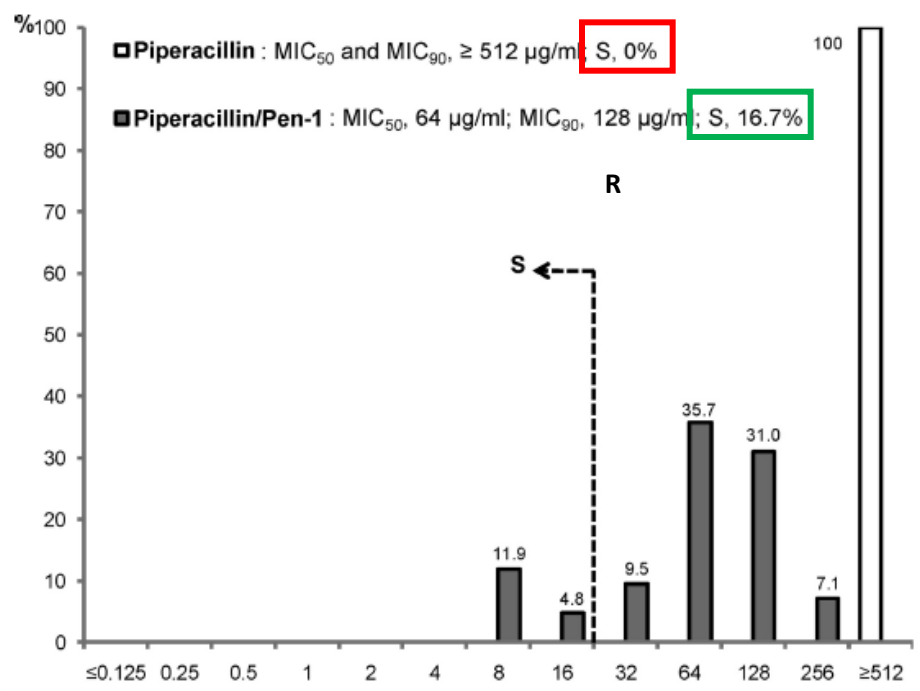


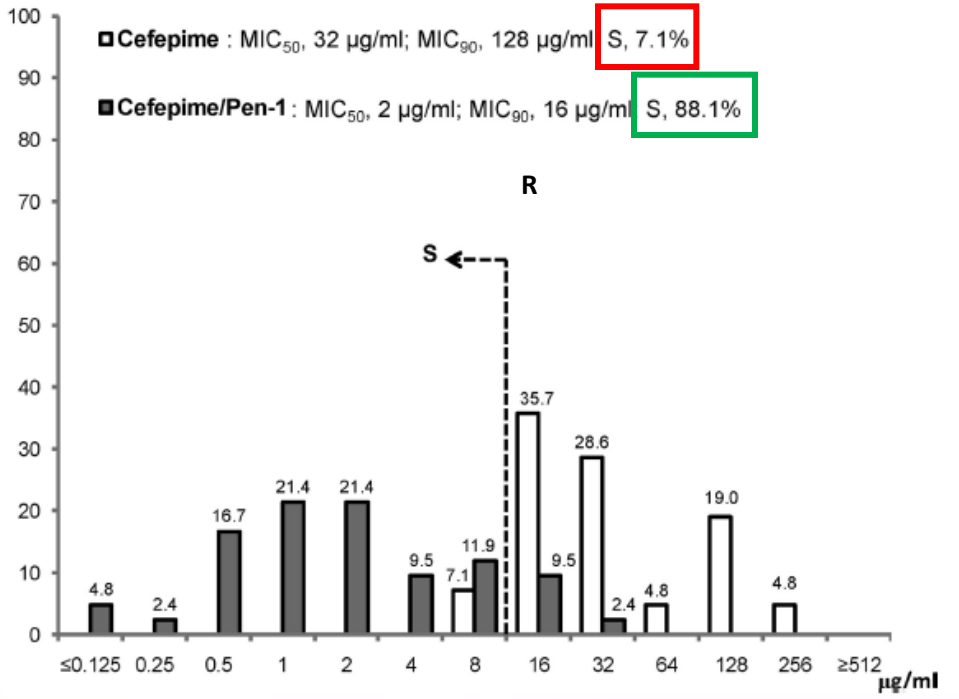
Tazobactam

資料來源：Proc Natl Acad Sci U S A. 2012; 109:11663-8

Penem-1

In vitro activity of penem-1+β-lactams against KPC *Klebsiella pneumonia*





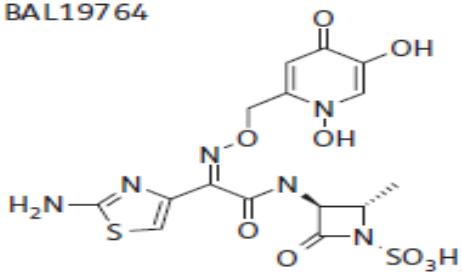
資料來源：Antimicrob Agents Chemother 2010; 54:1650-1

BAL30376

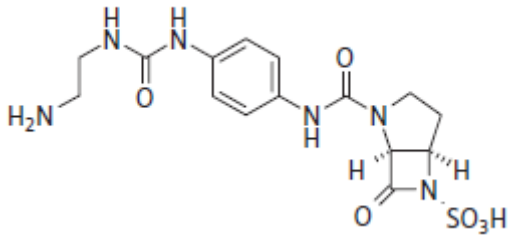
BAL30376

BAL197641 Monobactam	BAL298801 Monobactam	Clavulanate
Inhibit ESBL	Inhibit AmpC	Inhibit ESBL

BAL19764



BAL29880



Risk Factors For Acquiring

- No available literature of NDM-1 producing
- But many for KPC or other MBL-producing CRE
  - Host factors
  - Prior antibiotics exposure:
    - Broad-spectrum cephalosporin, Anti-pseudomonal penicillin, Fluoroquinolones
    - More consistant, carbapenems

資料來源：Daikos gL, et al. J Antimicrob Agents 2010; 65:784 – 8
Falagas ME, et al. J Antimicrob Agents 2007; 60:1124 – 30
Kwak YG, et al. Microb Drug Resist 2005; 11:165 – 9
Gasink BL, et al. Infect Control Hosp Epidemiol 2009; 30:1180 – 5

3.具有NDM-1 MBL腸道菌的預防與管制

預防與管制

- 一般民眾
  - 預定前往國外進行手術或侵入性治療，應確認該醫療機構是否採取合宜的感染管制措施。
  - 若曾在國外接受手術、侵入性治療或傷口處理，尤其是曾經前往印度、巴基斯坦者，回國後如有不適，應儘速就醫治療，並告知旅遊史與醫療史。
  - 不自行服用抗生素。
  - 遵照醫囑使用抗生素。

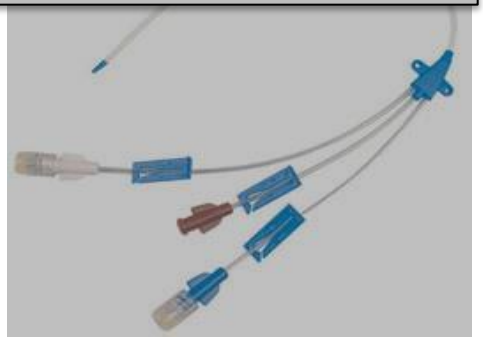
Hospital Prevention

1. Avoid underdose
2. Avoid prolonged antibiotics use.

Antimicrobial Stewardship

Contact Precautions

1. Colonization or infection with CRE
2. Exposure to antimicrobials (esp. quinolones)
3. Came from healthcare facility
4. Less than 3 months' elapsed time



Laboratory Notification

Hand Hygiene

Healthcare Personal Education

1. Clean sinks
2. Alcohol based hand
4. Immediate feedback

Patient and Staff Cohorting

Use of Devices

1. CVC, Foley, endotracheal tubes
2. Minimize device use

Take Home Message

- 抗藥性細菌的出現，是抗生素大量使用後必然出現的狀況。
  - Use antibiotics wisely.
- NDM-1 MBL-producing腸道菌可怕之處。
  - 可供使用以治療的抗生素十分有限。
  - 抗藥性基因可以水平傳播給其它細菌。
- 抗藥性細菌的防制之道。
  - 嚴格的接觸隔離措施。

課程結束