KANGWON NATIONAL UNIVERSITY

Comparative Assessment of β-Lactamases Produced by Multidrug Resistant Bacteria

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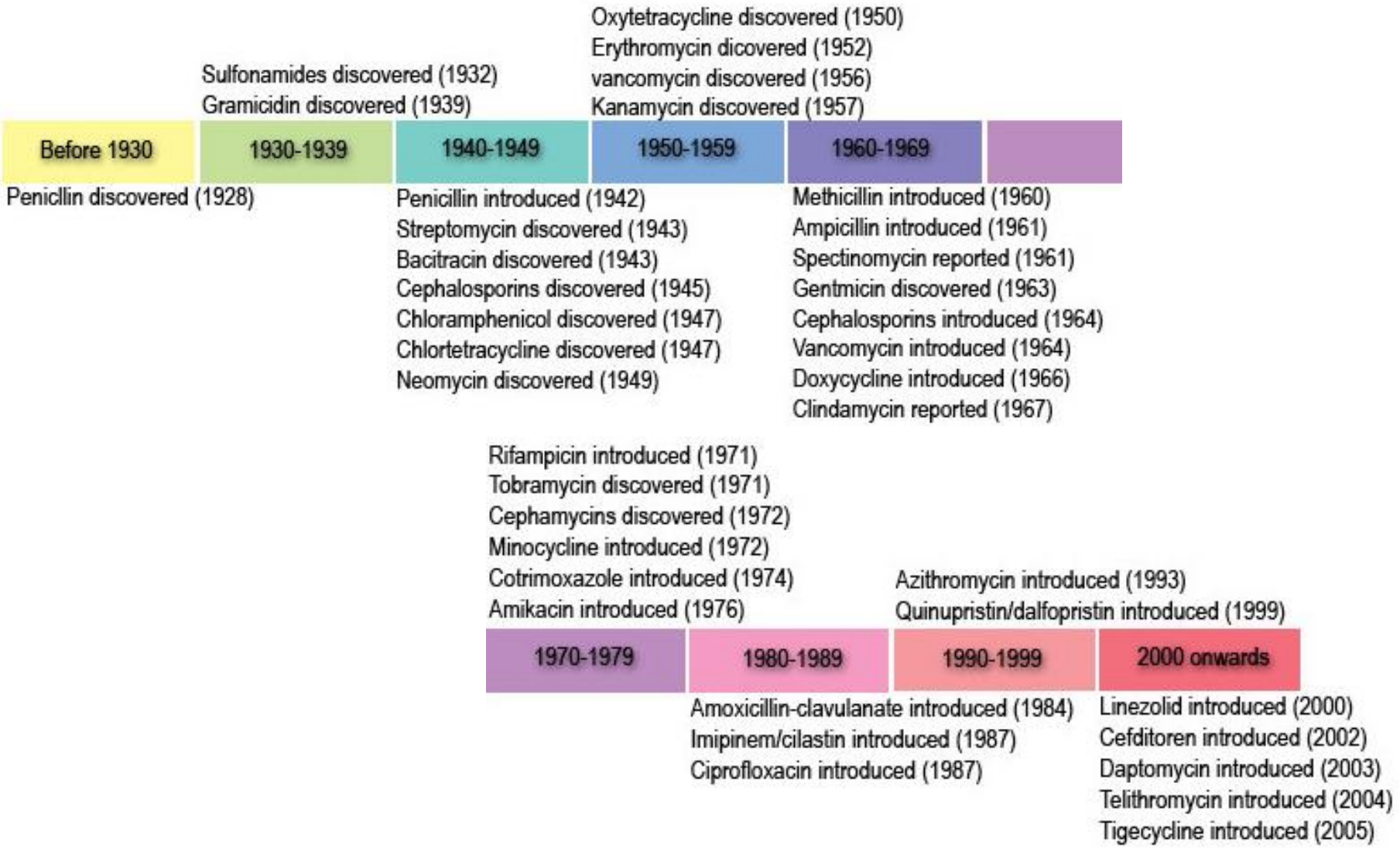
Antibiotic Development



Antibiotic Resistance Threats

Highly Resistance

Treatment failure to the last resort of medicine for gonorrhoea (third generation cephalosporin antibiotics) has been confirmed in at least 10 countries (Australia, Austria, Canada, France, Japan, Norway, Slovenia, South Africa, Sweden and the United Kingdom of Great Britain and Northern Ireland).

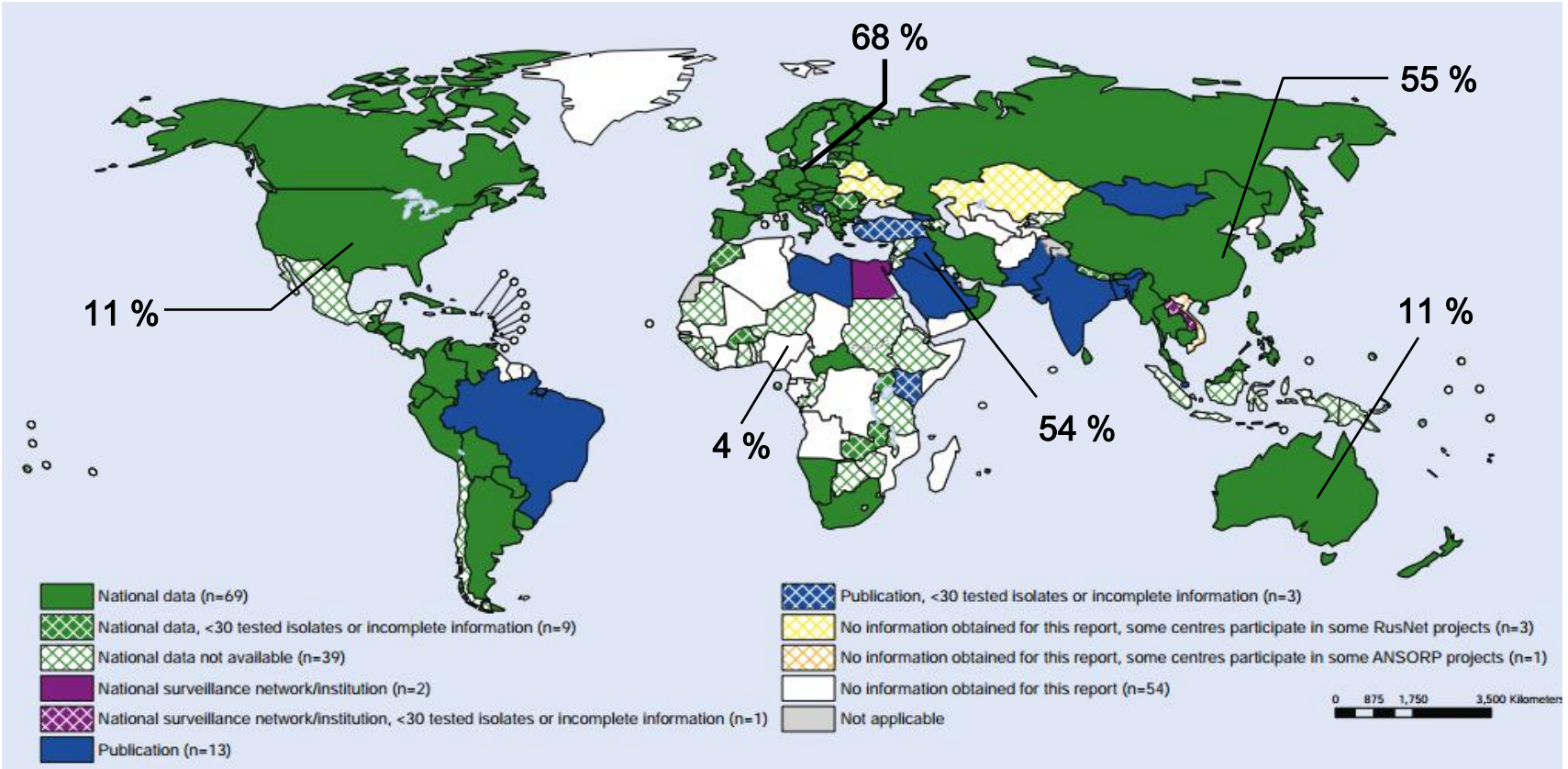
Pathogenic Bacteria

Resistance in *Klebsiella pneumoniae* – common intestinal bacteria that can cause life-threatening infections.
Resistance in *Escherichia coli* to one of the most widely used medicines for the treatment of urinary tract infections is very widespread.

World Spreading

Antibiotic resistance is present in every country. Antimicrobial resistant-microbes are found in people, animals, food, and the environment. They can spread between people and animals, and from person to person. Poor infection control, inadequate sanitary conditions and inappropriate food-handling encourage the spread of antimicrobial resistance.

Emergence of CR *Klebsiella pneumoniae*

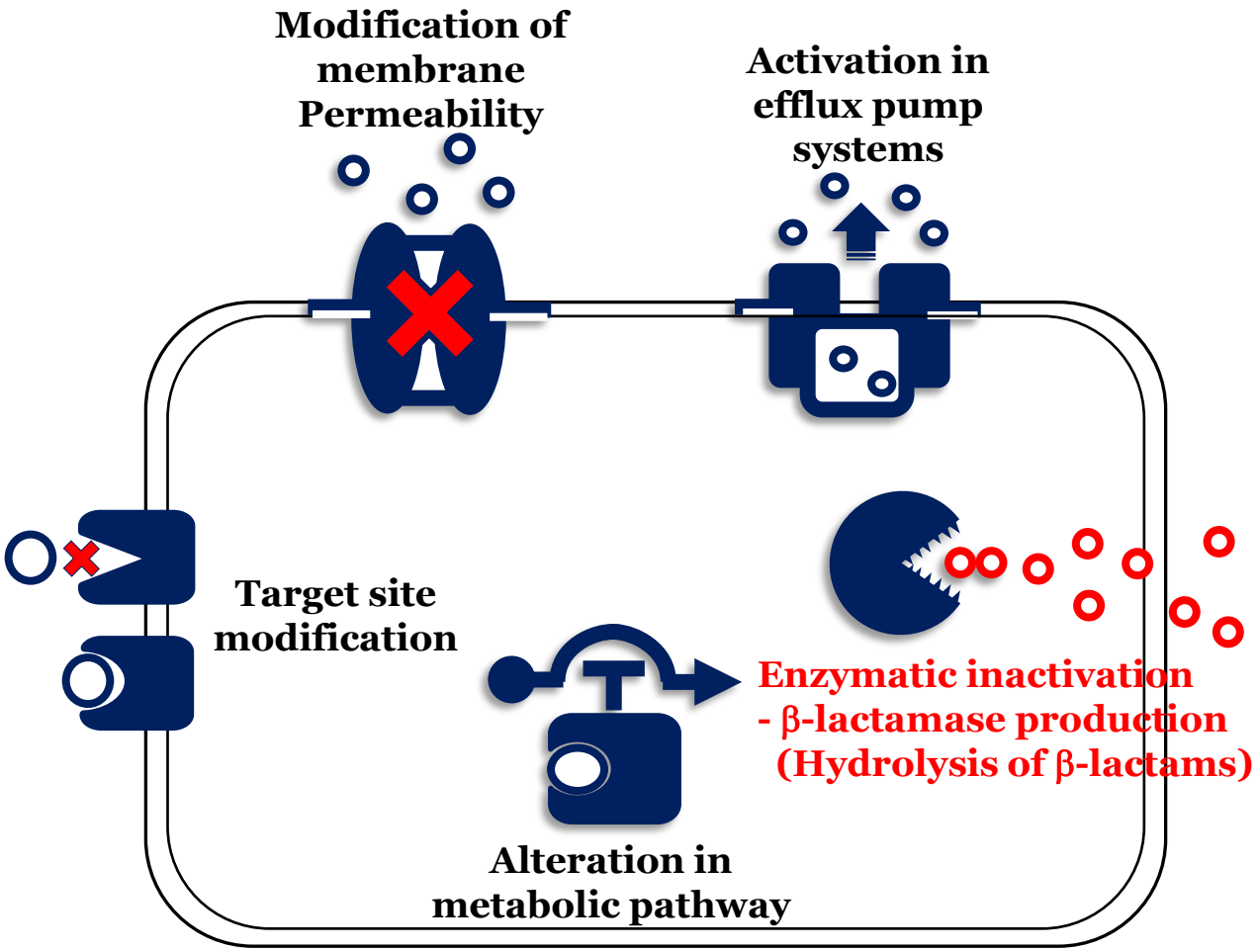


WHO, Antimicrobial Resistance Global Report 2014

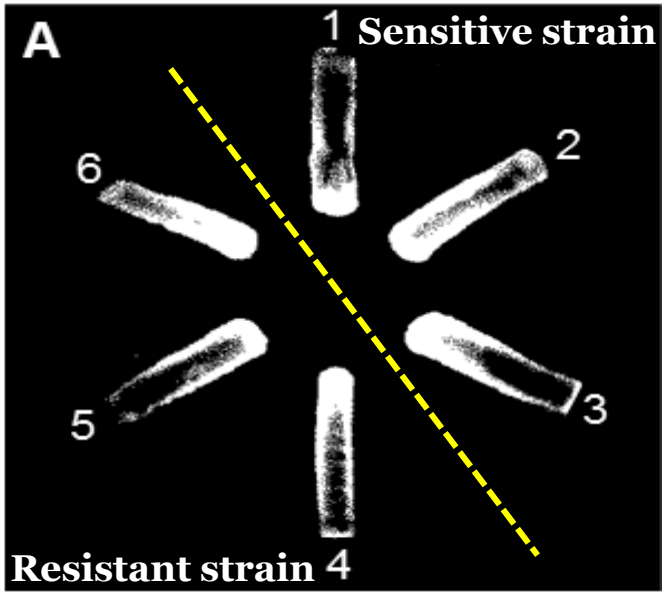
Klebsiella pneumoniae

- ***Klebsiella pneumoniae*** has become an emerging nosocomial pathogen
  - **Infectious diseases by *K. pneumoniae***: pneumonia, urinary tract infection, bacteremia, cholecystitis, osteomyelitis, meningitis, and thromobophlebitis
- **Antibiotic resistant** *K. pneumoniae* has been rapidly increased worldwide
- The increased resistance to β-lactam antibiotics in *K. pnuemoniae* is mainly caused by production of **extended spectrum β-lactamases (ESBL)**.
- Infection with ESBL producing *K. pneumoniae* leads to the failure of the disease treating.
- Moreover, **various types of β-lactamases** accelerate the emergence of multidrug-resistant *K. pneumoniae*.

Antibiotic Resistance Mechanisms

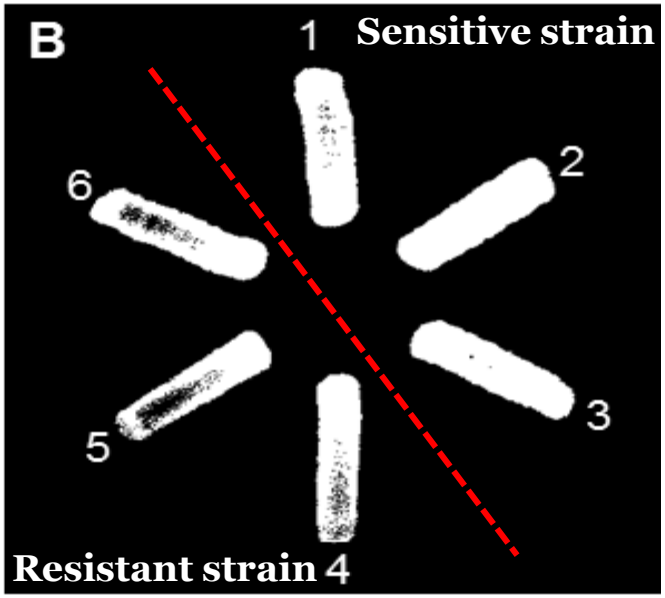


Efflux Pump Activity – EtBr accumulation assay



w/o PAβB

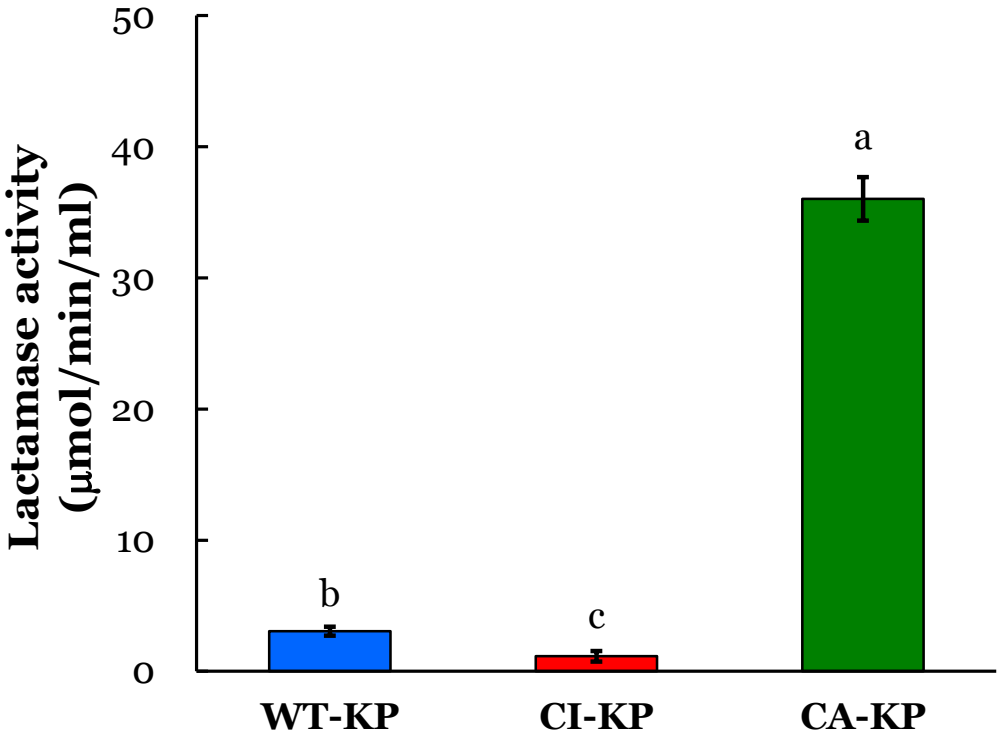
- EP activity
  (Low fluorescence intensity)



with PAβN

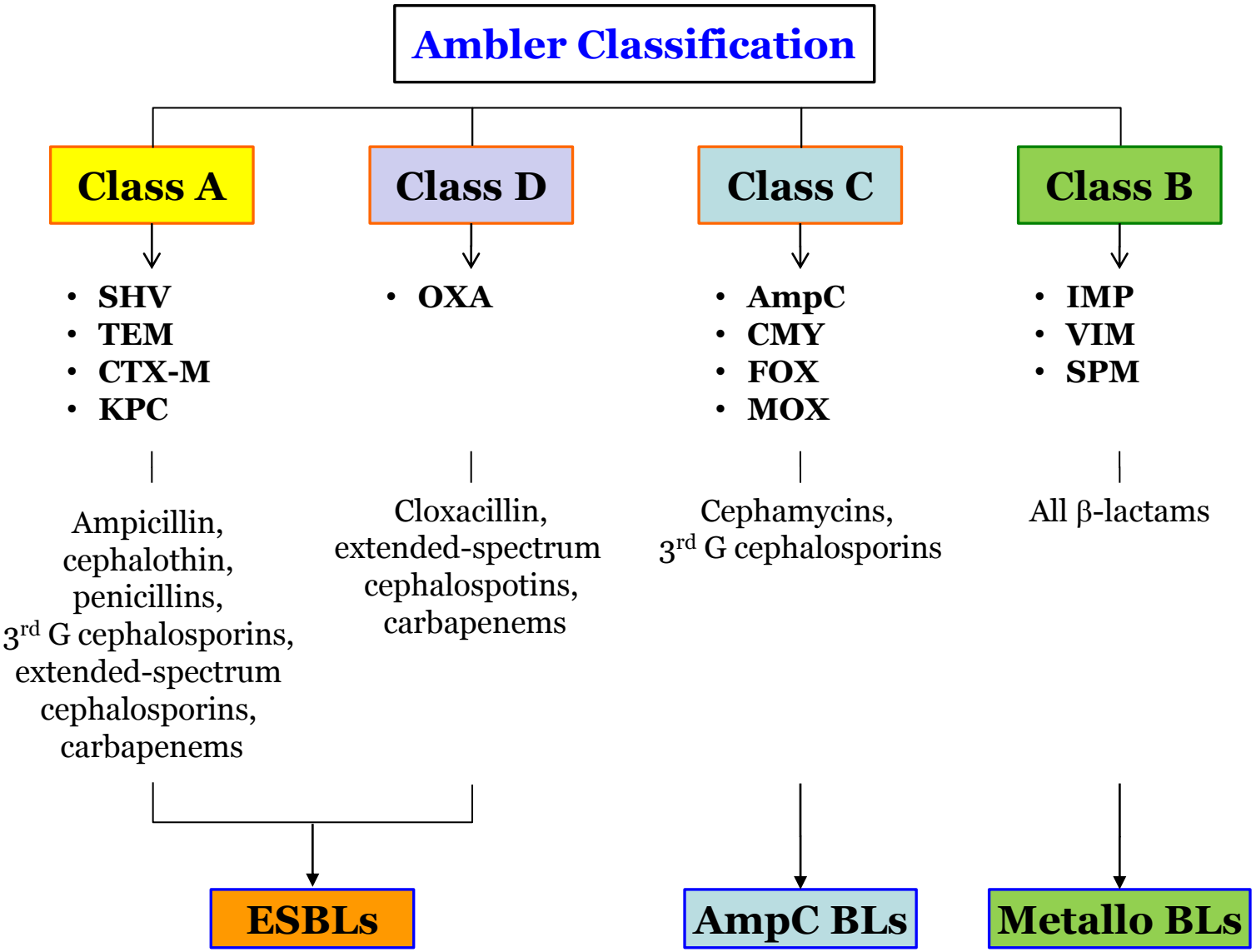
- Decrease in EP activity
- EPI-insensitive EP systems
  - Competitively expel substrates
  - Specific substrates: chloramphenicol, tetracycline, norfloxacin, nalidixic acid, and levofloxacin

β-Lactamase Activity – Nitrocefin hydrolysis assay



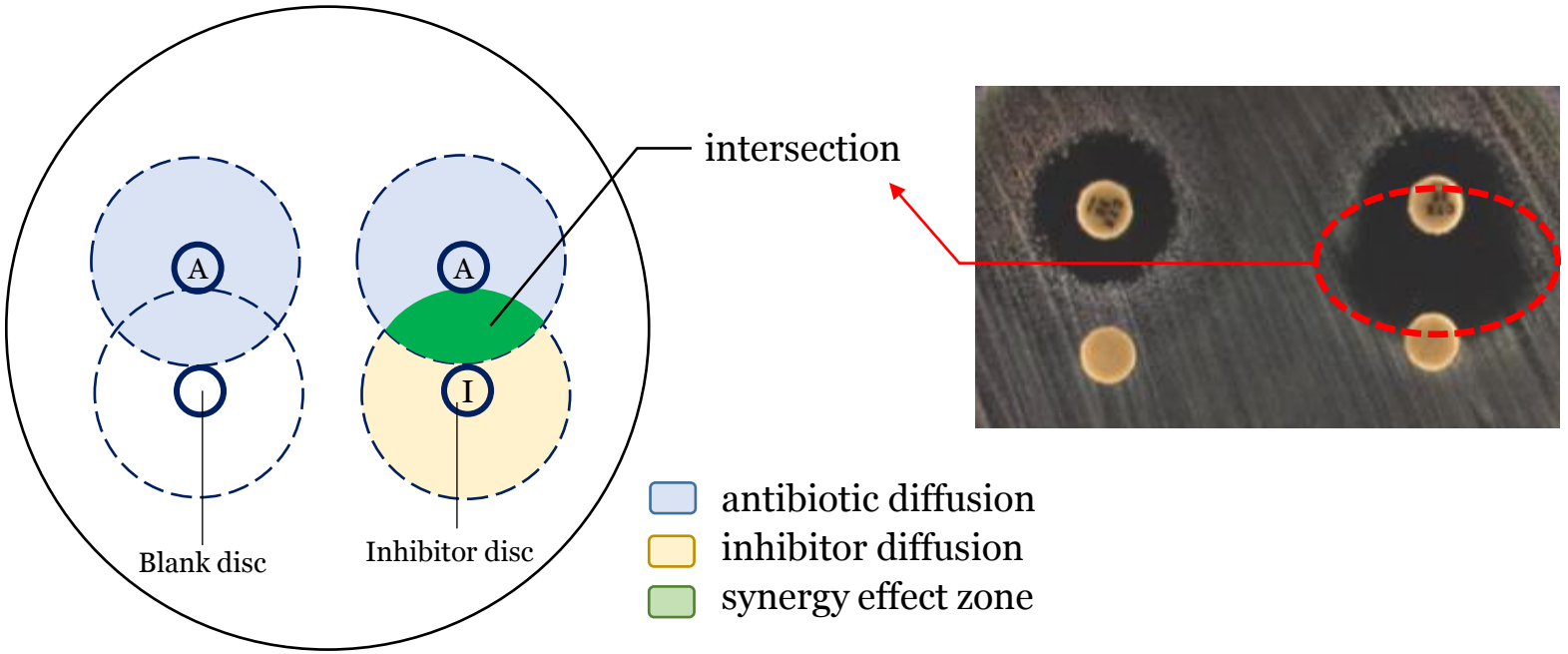
- Low β-lactamase activity
  - PBPs with low affinity for β-lactamases
- High β-lactamase activity
  - Production of β-lactamases

Classification of β-Lactamase



Experimental Approach

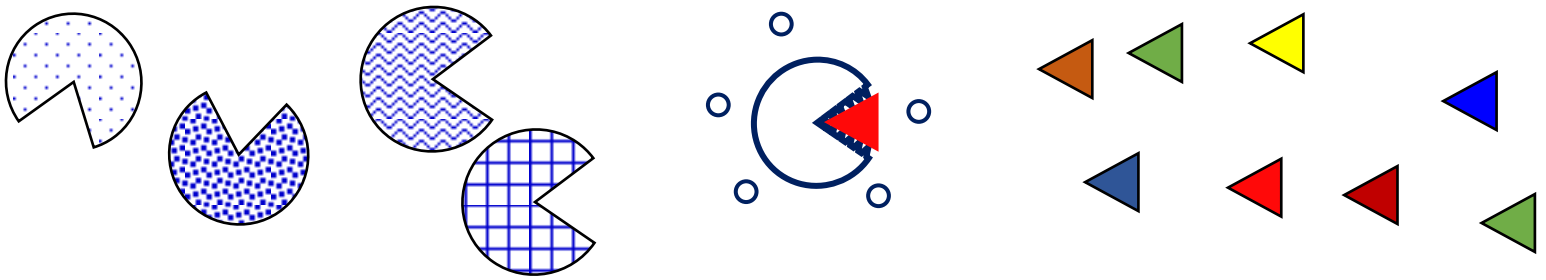
- Relationship between b-lactamase production and resistance phenotype to understand the resistance mechanisms in *K. pneumoniae*
  - WT-KP, CIP-KP, clinical isolates
  - Antibiotic susceptibility testing (with β-lactamase inhibitors)
    - BLIs: BLI-489, clavulanic acid, sulbactam, and tazobactam
  - Dual disc diffusion assay (with β-lactamase inhibitors)
  - PCR assay



β-Lactamase Inhibitors

- Structurally similar to β-lactam antibiotics
- Competitive inhibitor of β-lactamases
- Irreversible inhibition of β-lactam antibiotics
- Co-administrated with β-lactam antibiotics
  - Clavulanic acid + amoxicillin/ticarcillin
  - Sulbactam + ampicillin/cefoperazone
  - Tazobactam + piperacillin

β-Lactamases **β-Lactamase inhibitors**



Antibiotic Susceptibility Assay

MICs (μg/mL) of *K. pneumoniae* strains were determined in presence and absence of β-lactamase inhibitors

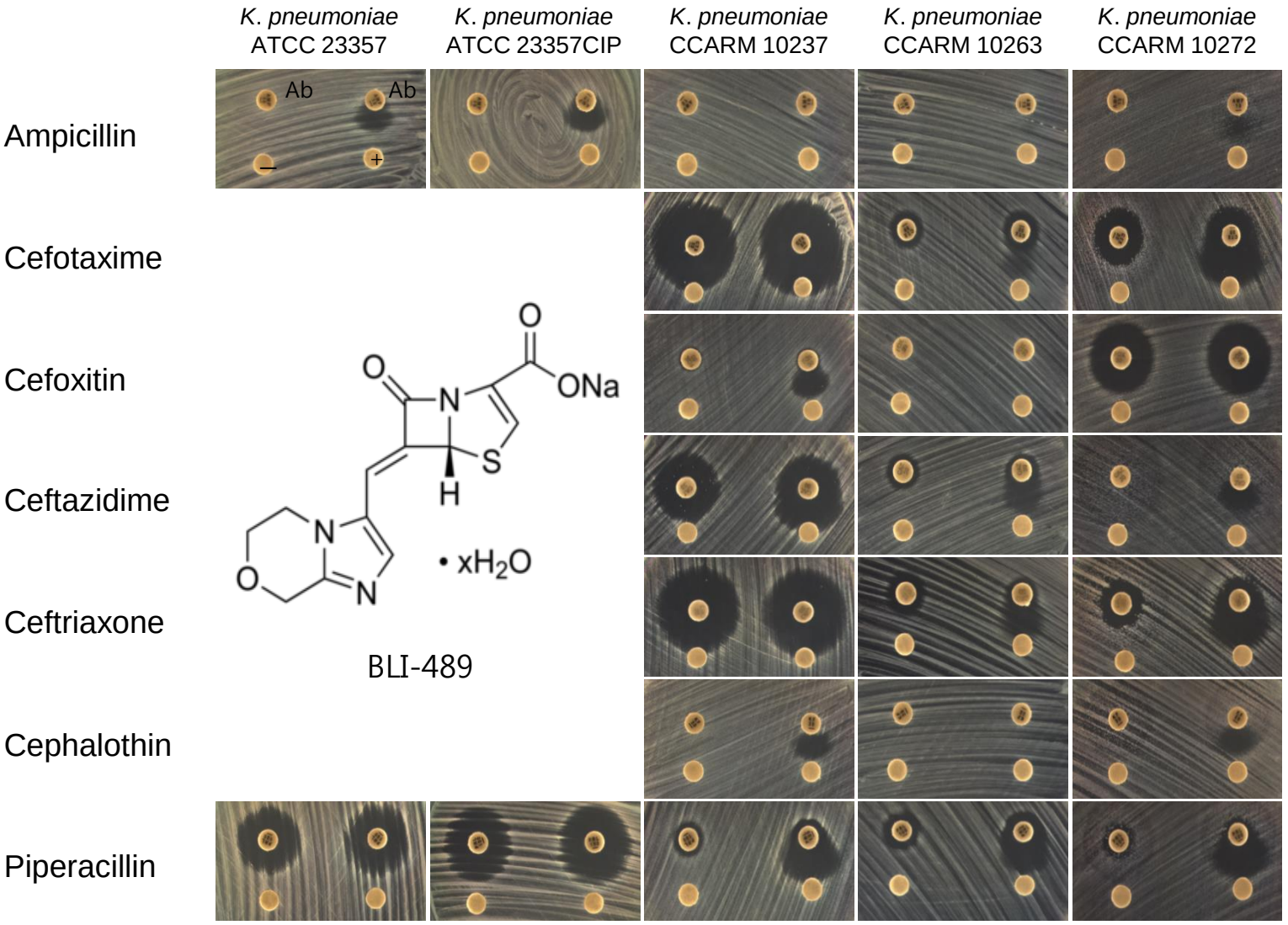
Antibiotic**	*K. pneumoniae* ATCC 23357					*K. pneumoniae* ATCC 23357-CIP					*K. pneumoniae* CCARM 10237					*K. pneumoniae* CCARM 10263					*K. pneumoniae* CCARM 10272				
	CO	BL	CA	SB	TB	CO	BL	CA	SB	TB	CO	BL	CA	SB	TB	CO	BL	CA	SB	TB	CO	BL	CA	SB	TB
AMP	>512 (R)†	8 (S)	8 (S)	128 (R)	16 (R)	>512 (R)	16 (R)	16 (R)	64 (R)	32 (R)	>512 (R)	512 (R)	>512 (R)	>512 (R)	>512 (R)	>512 (R)	256 (R)	>512 (R)	>512 (R)	>512 (R)	>512 (R)	128 (R)	32 (R)	>512 (R)	>512 (R)
CFT	0.3 (S)	0.25 (S)	0.25 (S)	0.15 (S)	0.25 (S)	0.5 (S)	0.5 (S)	0.25 (S)	0.25 (S)	0.5 (S)	64 (R)	0.5 (S)	32 (R)	32 (R)	64 (R)	512 (R)	32 (R)	128 (R)	128 (R)	512 (R)	512 (R)	<0.5 (S)	0.5 (S)	64 (R)	128 (R)
CFX	8 (S)	8 (S)	8 (S)	8 (S)	16 (R)	16 (R)	16 (R)	16 (R)	16 (R)	32 (R)	512 (R)	128 (R)	512 (R)	512 (R)	512 (R)	>512 (R)	>512 (R)	>512 (R)	>512 (R)	>512 (R)	16 (R)	16 (R)	16 (R)	16 (R)	16 (R)
CFZ	0.5 (S)	0.5 (S)	0.25 (S)	0.25 (S)	0.5 (S)	0.5 (S)	1 (S)	0.25 (S)	0.5 (S)	0.5 (S)	256 (R)	2 (R)	128 (R)	128 (R)	256 (R)	>512 (R)	16 (R)	64 (R)	128 (R)	512 (R)	>512 (R)	64 (R)	2 (R)	>512 (R)	>512 (R)
CFA	0.3 (S)	0.13 (S)	0.25 (S)	0.25 (S)	0.13 (S)	0.3 (S)	0.25 (S)	0.25 (S)	0.25 (S)	0.25 (S)	32 (R)	0.25 (S)	16 (R)	16 (R)	64 (R)	>512 (R)	16 (R)	128 (R)	128 (R)	512 (R)	512 (R)	1 (S)	0.25 (S)	128 (R)	256 (R)
CEP	16 (R)	16 (R)	8 (S)	16 (R)	16 (R)	32 (R)	16 (R)	16 (R)	16 (R)	32 (R)	>512 (R)	256 (R)	>512 (R)	>512 (R)	>512 (R)	>512 (R)	>512 (R)	>512 (R)	>512 (R)	>512 (R)	>512 (R)	32 (R)	8 (S)	>512 (R)	>512 (R)
IMP	2 (S)	4 (I)	4 (I)	2 (S)	1 (S)	2 (S)	2 (S)	2 (S)	0.5 (S)	2 (S)	4 (I)	1 (S)	2 (S)	2 (S)	1 (S)	2 (S)	0.5 (S)	2 (S)	4 (I)	2 (S)	2 (S)	1 (S)	2 (S)	2 (S)	2 (S)
MER	0.3 (S)	0.25 (S)	0.25 (S)	0.25 (S)	0.13 (S)	0.3 (S)	0.25 (S)	0.13 (S)	0.25 (S)	0.13 (S)	1 (S)	0.13 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	0.5 (S)	1 (S)	2 (S)	0.5 (S)	0.5 (S)	0.13 (S)	0.25 (S)	0.5 (S)
PIP	32 (R)	4 (S)	4 (S)	8 (S)	8 (S)	32 (R)	8 (S)	8 (S)	8 (S)	8 (S)	>512 (R)	8 (S)	256 (R)	>512 (R)	>512 (R)	>512 (R)	16 (R)	32 (R)	256 (R)	512 (R)	>512 (R)	16 (R)	4 (S)	>512 (R)	>512 (R)

* CO, control; BLI-489, BL; clavulanate, CA; sulbactam, SB; tazobactam, TB.

** AMP, ampicillin; CFT, cefotaxime; CFX, cefoxitin; CFZ, ceftazidime; CFA, ceftriaxone; CEP, cephalothin; IMI, imipenem; MER, meropenem; PIP, piperacillin.

† S, susceptible; I, intermediate; R, resistant.

BLI-489


K. pneumoniae ATCC 23357
K. pneumoniae ATCC 23357CIP
K. pneumoniae CCARM 10237
K. pneumoniae CCARM 10263
K. pneumoniae CCARM 10272
Ampicillin
Cefotaxime
Cefoxitin
Ceftazidime
Ceftriaxone
Cephalothin
Piperacillin
Ab
Ab
–
+
O
O
N
ONa
S
H
N
O
N
• xH2O
BLI-489

BLI-489

- Increased susceptibility
  - CCARM10237: cefotaxime, cefoxitin, ceftazidime, ceftriaxone, cephalothin, and piperacillin

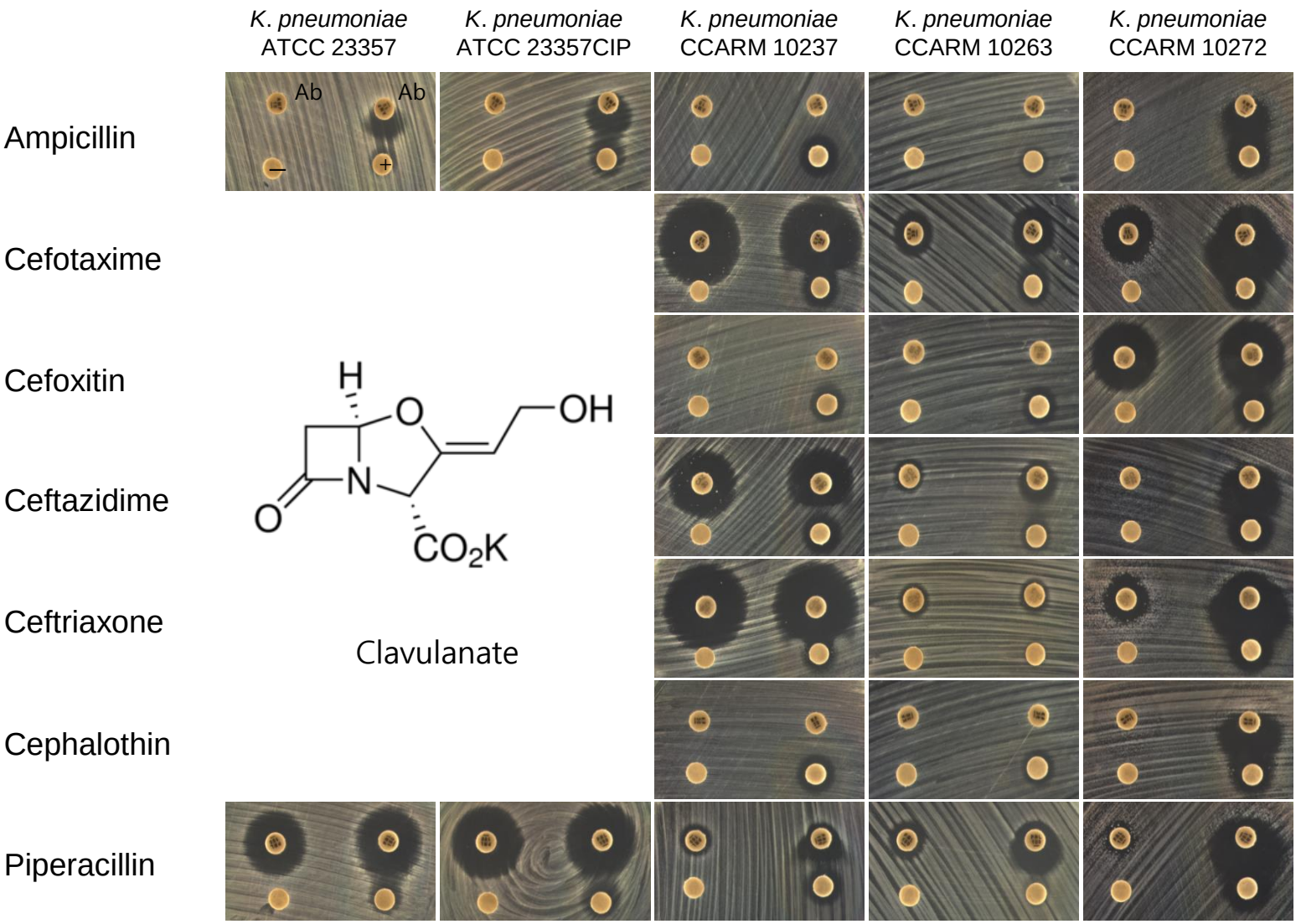
  (Hydrolysis inhibited by BLI-489)

  - CCARM10263: cefotaxime, ceftazidime, ceftriaxone, and piperacillin

- No susceptibility changes
  - CCARM10263: ampicillin, cefoxitin, and cephalothin

	BLI-489		BLI-489		BLI-489		BLI-489		BLI-489	
	−	+	−	+	−	+	−	+	−	+
	6.00	9.53	6.00	12.64	6.00	6.00	6.00	6.00	6.00	7.92
					24.47	26.85	10.34	17.75	16.08	22.69
					6.82	13.31	6.00	6.00	19.61	21.21
					20.57	23.51	9.49	15.71	6.00	10.15
					24.60	24.89	10.92	17.15	13.35	17.85
					6.00	8.17	6.00	6.00	6.00	9.73
	18.62	19.27	19.22	22.29	10.49	18.15	10.51	17.21	8.59	17.19

Clavulanic acid

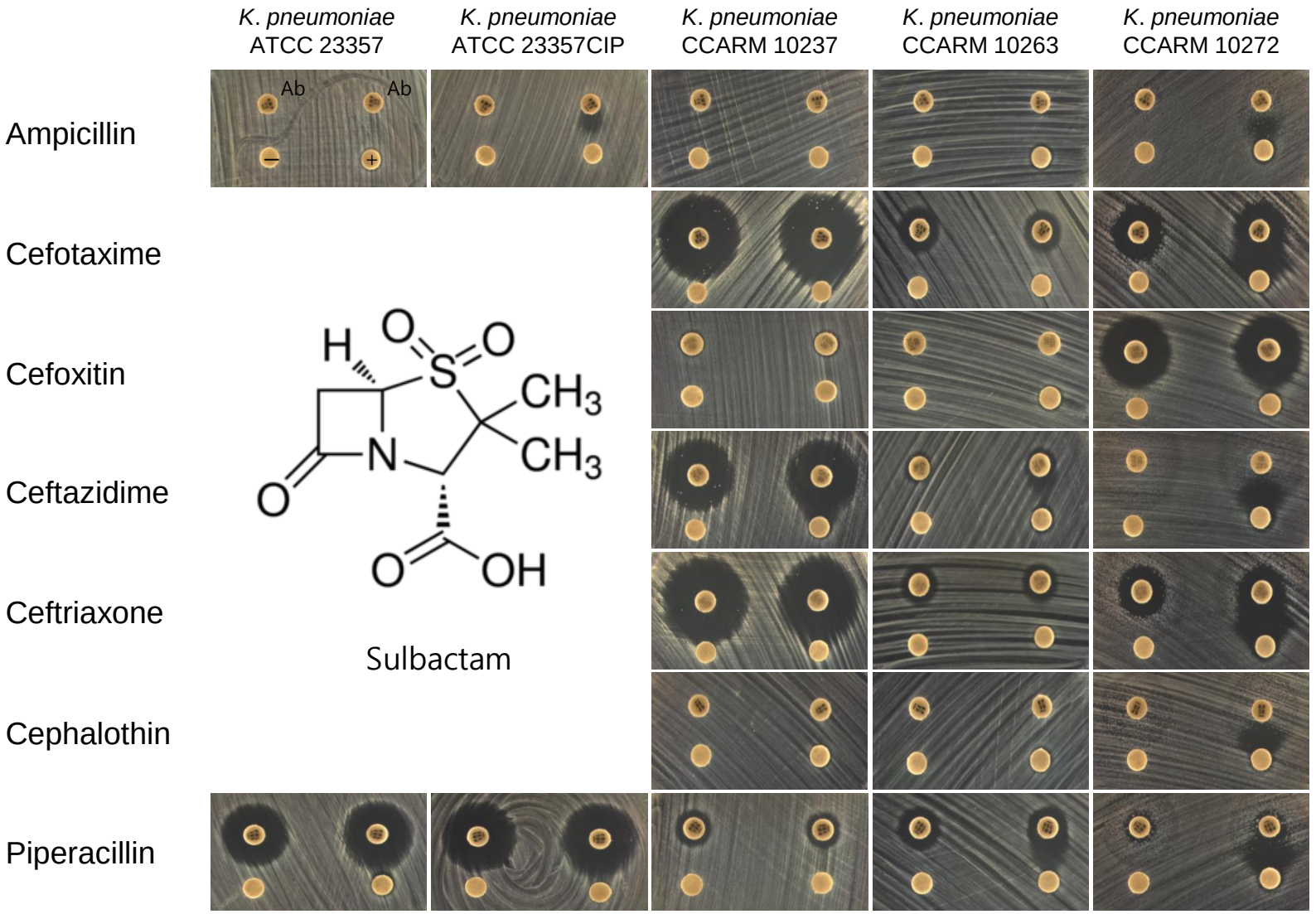


Clavulanic acid

- Increased susceptibility
  - ATCC23357, ATCC-CIP, and CCARM10272: ampicillin and piperacillin
  - CCARM10237: cefotaxime and piperacillin
- No susceptibility changes
  - CCARM10267 and CCARM10263: ampicillin, cefoxitin, and cephalothin

[redacted]	[redacted]		[redacted]		[redacted]		[redacted]		[redacted]	
[redacted]	Clav.		Clav.		Clav.		Clav.		Clav.	
[redacted]	−	+	−	+	−	+	−	+	−	+
[redacted]	6.00	11.40	6.00	13.14	6.00	6.00	6.00	6.00	6.00	23.39
[redacted]					24.80	32.57	13.73	17.89	16.19	29.96
[redacted]					6.00	6.00	6.00	6.00	18.67	31.89
[redacted]					18.66	16.94	9.13	13.19	6.00	23.76
[redacted]					25.29	24.33	9.22	8.96	13.19	29.39
[redacted]					6.00	6.00	6.00	6.00	6.00	22.82
[redacted]	17.40	29.90	21.67	28.10	8.89	12.58	10.98	16.55	8.81	26.45

Sulbactam


K. pneumoniae ATCC 23357
K. pneumoniae ATCC 23357CIP
K. pneumoniae CCARM 10237
K. pneumoniae CCARM 10263
K. pneumoniae CCARM 10272
Ampicillin
Cefotaxime
Cefoxitin
Ceftazidime
Ceftriaxone
Cephalothin
Piperacillin
Ab
Ab
–
+
Sulbactam

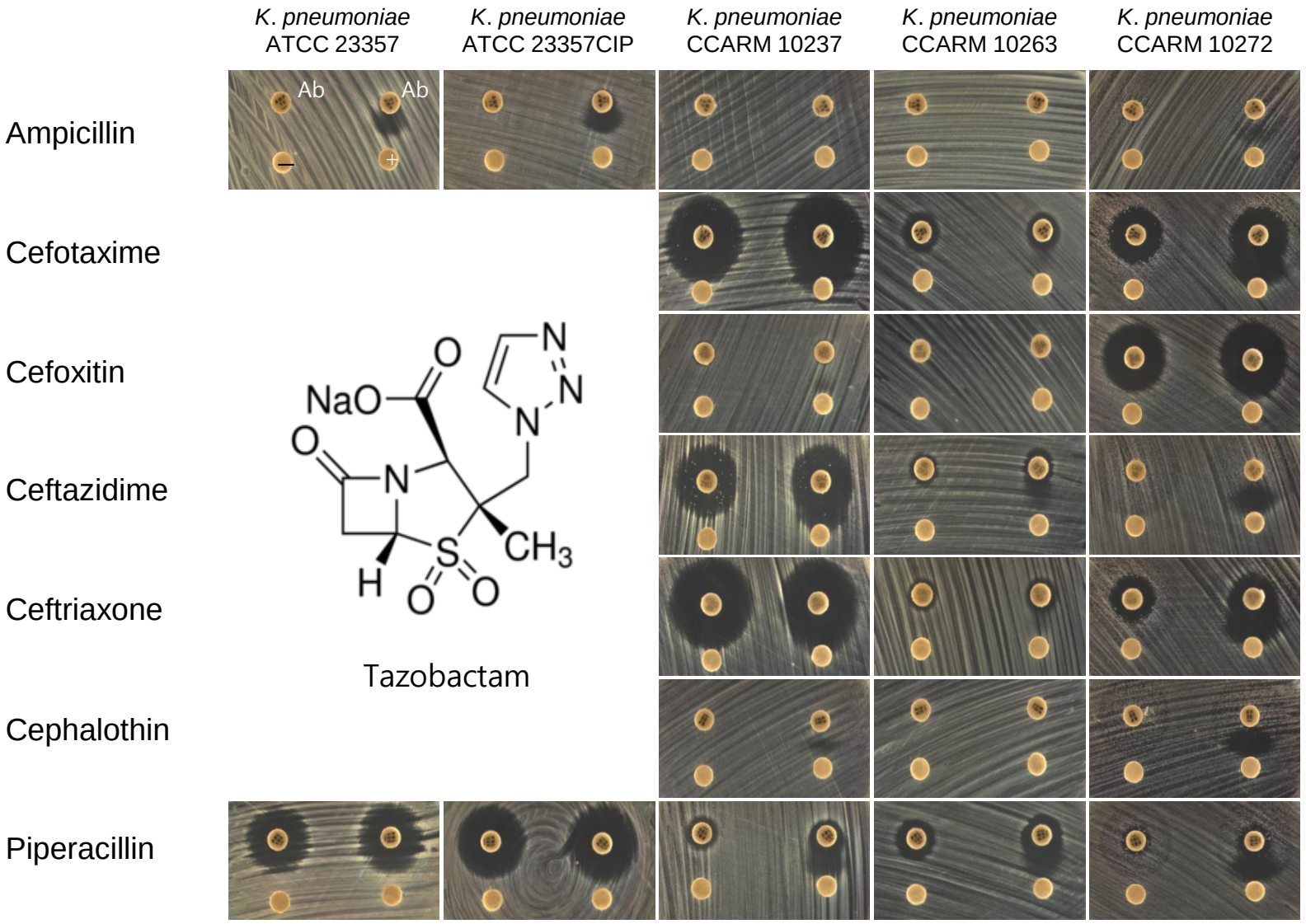
Sulbactam

- Increased susceptibility
  - CCARM10272: ampicillin, cefotaxime, ceftazidime, ceftriaxone, cephalothin, and piperacillin

- No susceptibility changes
  - ATCC23357 and ATCC-CIP: ampicillin, cefotaxime, cefoxitin, ceftazidime, ceftriaxone, and cephalothin

	Sulb.		Sulb.		Sulb.		Sulb.		Sulb.	
	−	+	−	+	−	+	−	+	−	+
	6.00	6.00	6.00	6.51	6.00	6.00	6.00	6.00	6.00	7.55
					25.45	27.17	11.32	11.45	15.85	22.60
					6.00	6.00	6.00	6.00	20.50	21.45
					20.35	25.87	8.54	15.11	6.00	15.13
					24.58	28.72	10.35	11.76	14.91	25.38
					6.00	6.00	6.00	6.00	6.00	13.02
	18.01	20.20	20.40	20.44	10.47	9.99	12.88	19.58	8.46	20.71

Tazobactam


K. pneumoniae ATCC 23357
K. pneumoniae ATCC 23357CIP
K. pneumoniae CCARM 10237
K. pneumoniae CCARM 10263
K. pneumoniae CCARM 10272
Ampicillin
Cefotaxime
Cefoxitin
Ceftazidime
Ceftriaxone
Cephalothin
Piperacillin
Ab
Ab
–
+
NaO
O
O
N
N
N
S
CH3
H
O
O
Tazobactam

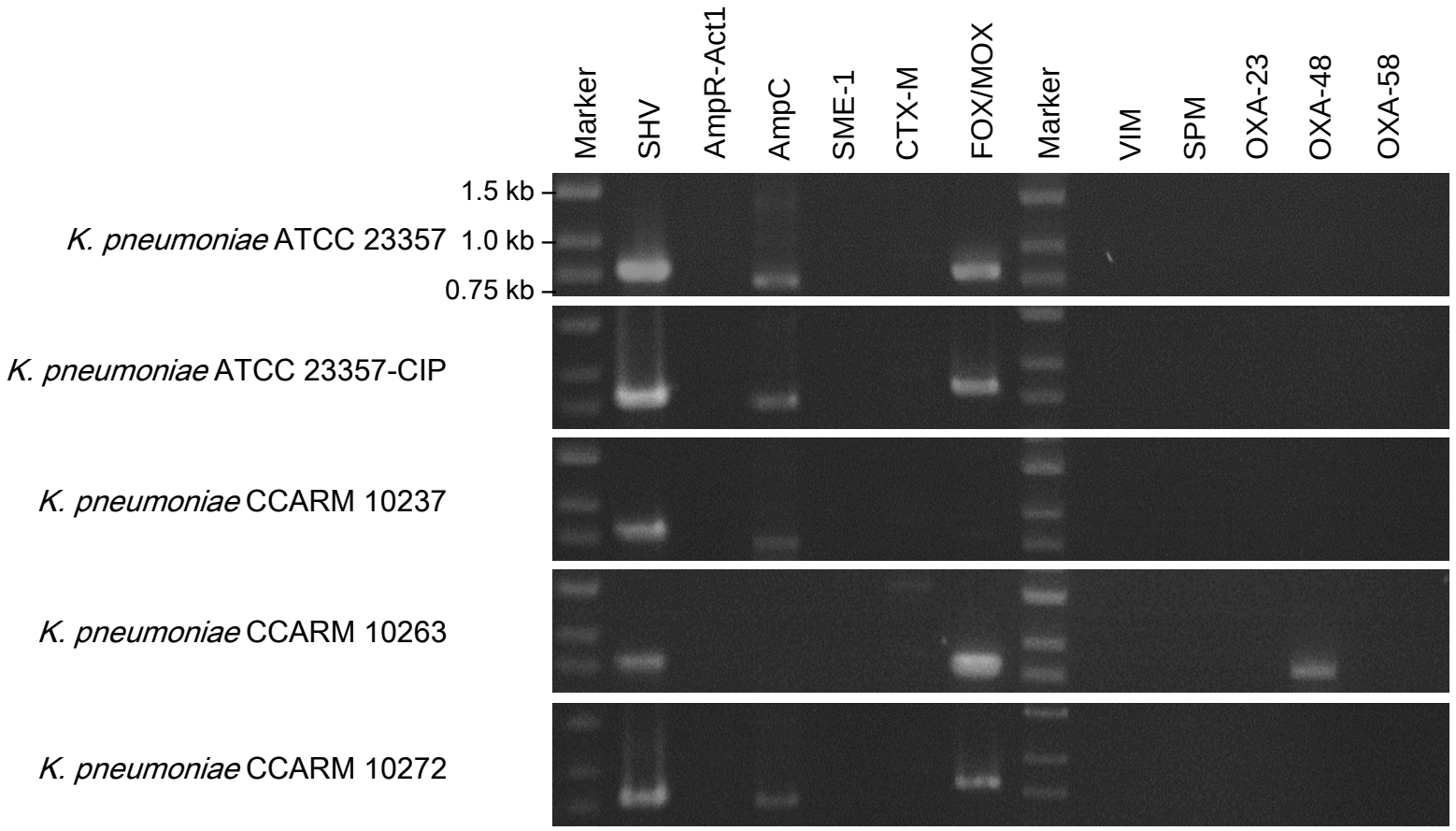
Tazobactam

- Increased susceptibility
  - CCARM10237, CCARM10263, and CCARM10272: ceftazidime and piperacillin

- No susceptibility changes
  - CCARM10237: ampicillin and cefoxitin
  - CCARM10262: ceftazidime, ceftriaxone, and cephalothin

	Tazo.		Tazo.		Tazo.		Tazo.		Tazo.	
	−	+	−	+	−	+	−	+	−	+
	6.00	8.65	6.00	9.50	6.00	6.00	6.00	6.00	6.00	6.00
					24.76	30.62	11.00	10.32	15.19	21.07
					6.00	6.00	6.00	6.00	17.41	20.66
					19.14	24.40	9.46	15.41	6.00	12.50
					26.26	24.89	9.16	9.36	13.79	20.88
					6.00	4.43	6.00	6.00	6.00	8.75
	17.68	19.70	19.47	21.45	10.71	16.29	13.32	17.39	15.89	17.02

Genotypic Characteristics of BLs



Agarose gel electrophoresis of β-lactamases extracted from *K. pneumoniae* selected in this study

- Predominant β-lactamases: plasmid-mediated ESBLs and chromosomally-mediated AmpC

Summary

- The production of various types of β-lactamase is considered a major mechanism responsible for the resistance to β-lactam antibiotics

- The MIC values of β-lactams against *K. pneumoniae* ATCC 23357, ciprofloxacin-induced *K. pneumoniae* ATCC 23357, *K. pneumoniae* CCARM 10237, *K. pneumoniae* CCARM 10263, and *K. pneumoniae* CCARM 10272 corresponded with the production of β-lactamases obtained from the dual disc diffusion assay.

- The constitute class A (SHV) and inducible class C (AmpC and FOX/MOX) were confirmed in *K. pneumoniae* ATCC 23357, ciprofloxacin-induced *K. pneumoniae* ATCC 23357, and *K. pneumoniae* CCARM 10272. The class A (SHV), class C(FOX/MOX), and class D(OXA-48) were confirmed in *K. pneumoniae* CCARM 10263.

- **The β-lactam resistance associated with the production of β-lactamases in *K. pneumoniae* strains can provide useful information for designing an infection control strategy in combination with appropriate β-lactamase inhibitors.**

Thank you!