Shiraz University of Medical Sciences

In The Name of God

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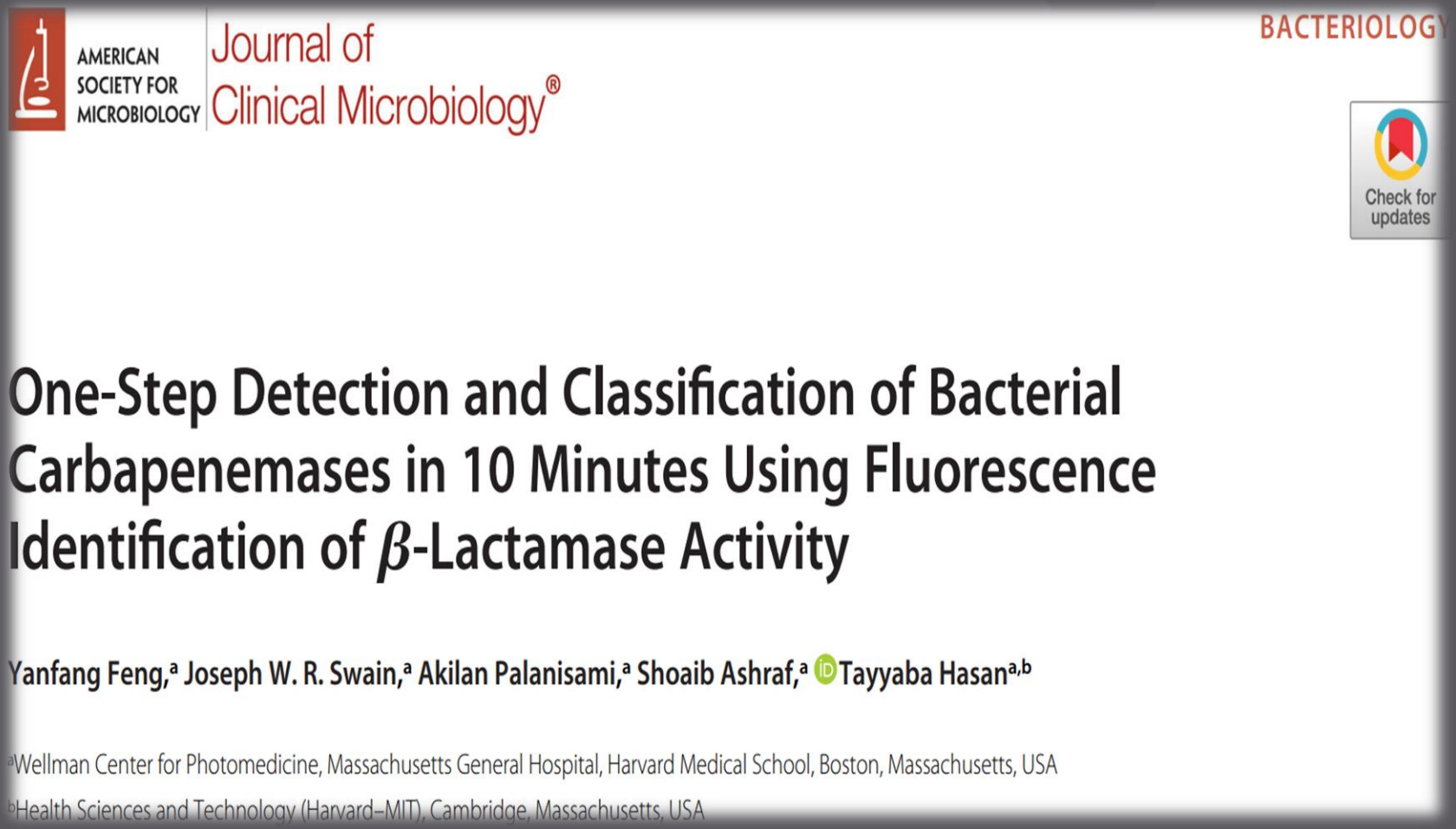

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BACTERIOLOGY

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One-Step Detection and Classification of Bacterial Carbapenemases in 10 Minutes Using Fluorescence Identification of β-Lactamase Activity

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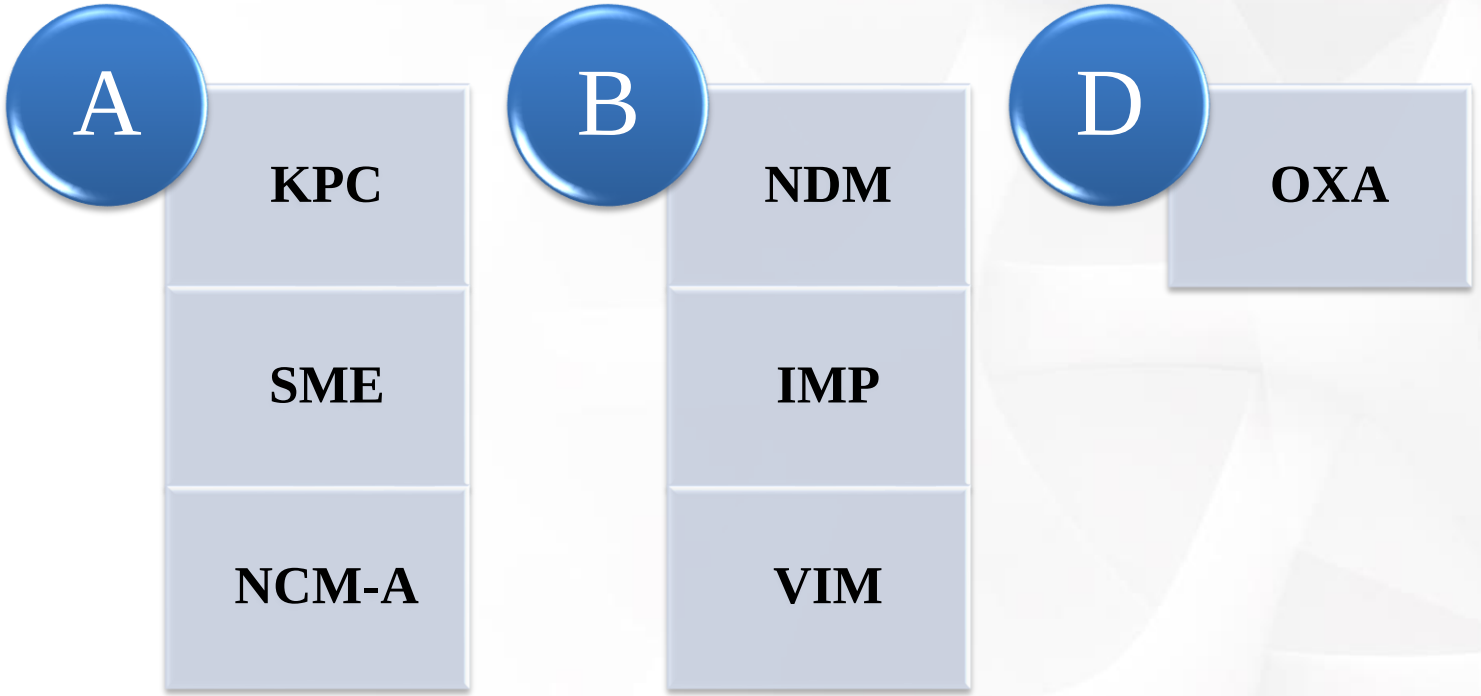
Introduction

- As a potent β-lactamase, carbapenemase can degrade almost all β-lactam antimicrobial drugs including the **carbapenems**

- The global prevalence of carbapenemases has been of great concern

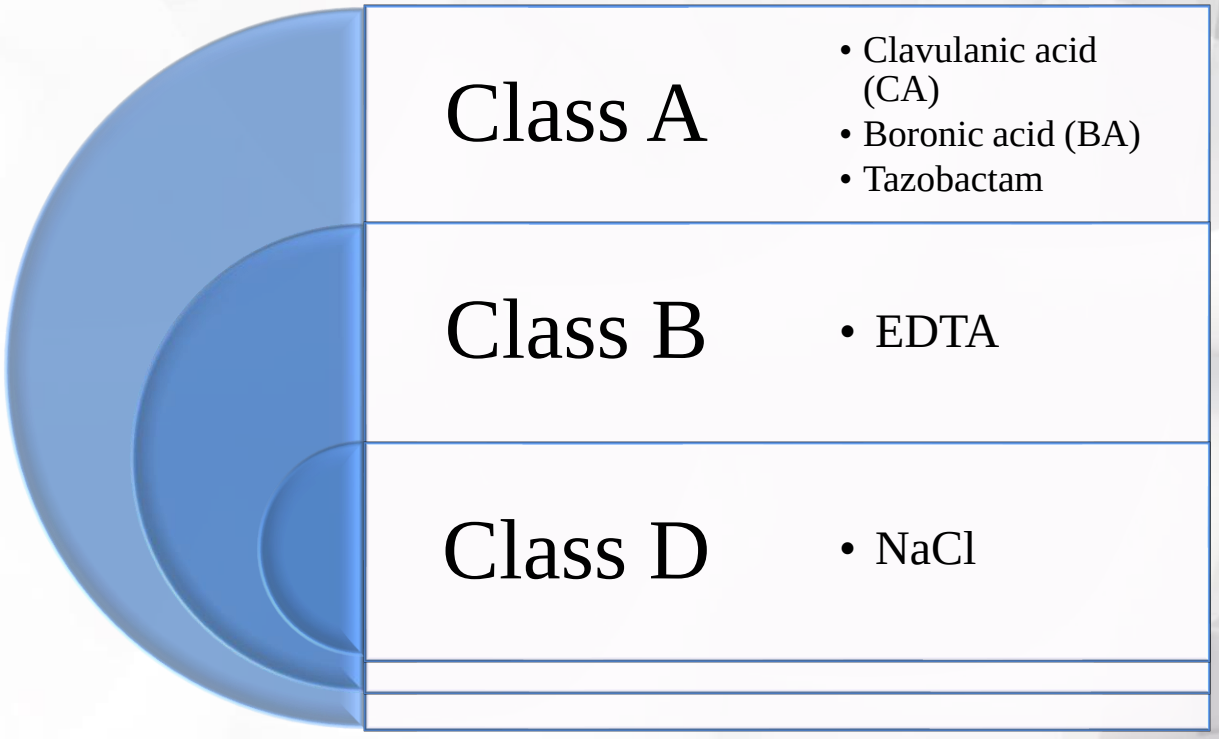
Introduction

- Based on their molecular structures, carbapenemases can be divided into three classes:



Introduction

- Different molecular classes of carbapenemases vary significantly in their susceptibilities toward different **β-lactamase inhibitors** (BLIs)



Introduction

- Timely carbapenemase detection and classification are **still challenging** for microbiology laboratories
- Phenotypic assays require at least **18 to 24** h despite being inexpensive and easily established

Introduction

- Relatively fast turnaround times (**15 min to 2 h**) is recorded for:
  1. Immunochromatographic lateral flow assays
  2. Molecular tests of carbapenemase genes

But there are some problems:
1. Costly
2. Generally available only for the most common carbapenemases

Introduction

- The recently developed (2012) **Carba NP test** and variants are elegant solutions & take only **2 h**

➢ Low **sensitivity** for OXA-48-like carbapenemases

➢ Subjective **interpretation** in color changes are concerning

Introduction

A recently developed fluorogenic assay:

- ✓ Synthesizing carbapenem-based fluorogenic probe
- ✓ Using the carbapenem moiety as a substrate for carbapenemases
- ✓ Allowing carbapenemases to be detected quantitatively and objectively in **90 min**

Introduction

Disadvantages:

1. Its speed is dictated by the initial **cell lysis** step

2. Often much more costly than the synthesis of probes based on other β-lactam drugs

3. This fluorogenic platform has not been developed and tested for carbapenemase molecular class characterization

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PMID: 32186503

Novel Rapid Test for Detecting Carbapenemase

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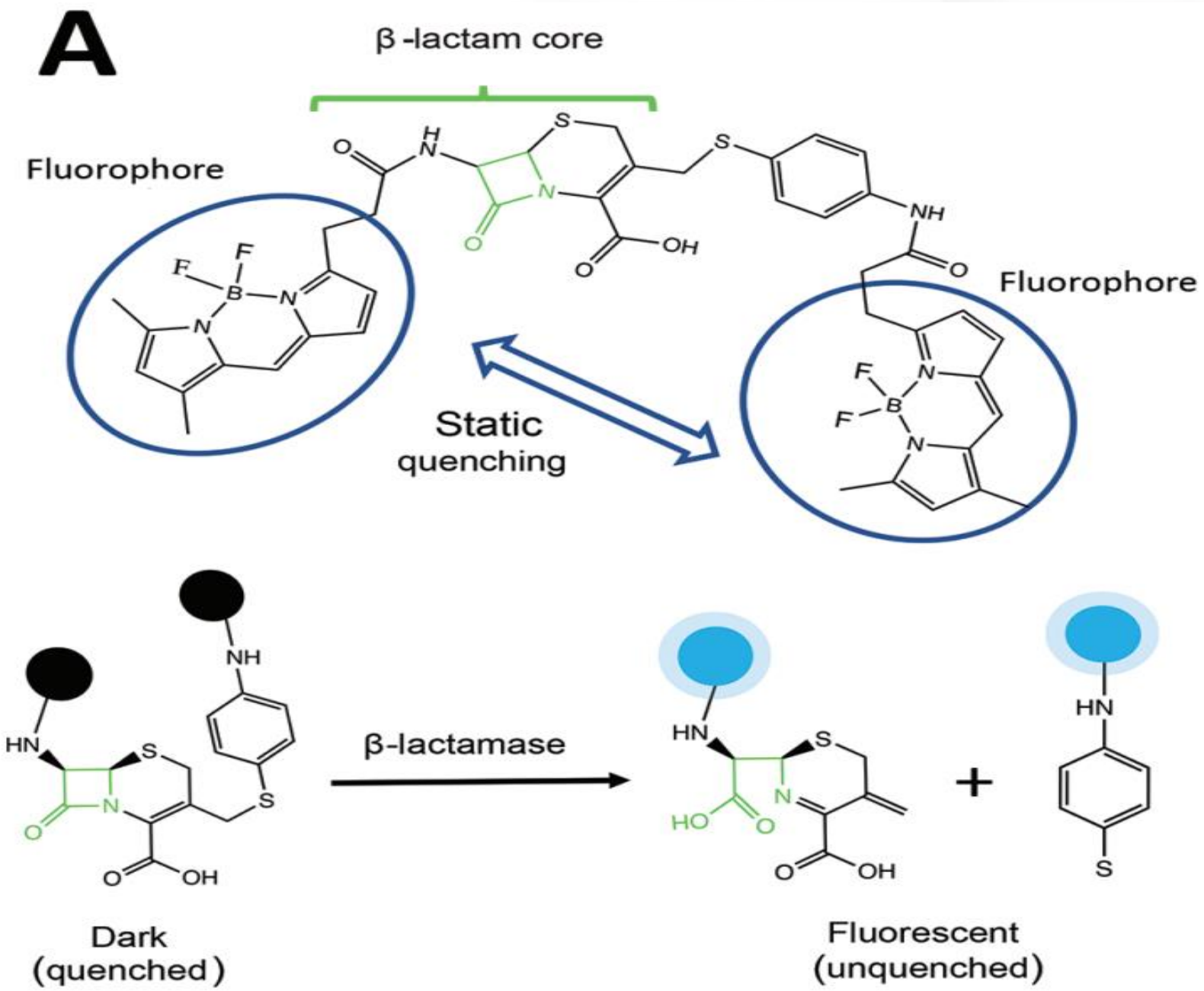
- **Yanfang Feng et al.** have developed a cephalosporin-based fluorescent probe (2020)

  - ✓ Known as **β-LEAF** (β-lactamase enzyme-activated fluorophore)

  - ✓ For the rapid fluorescence identification of β-lactamase activity (**FIBA**) in bacteria

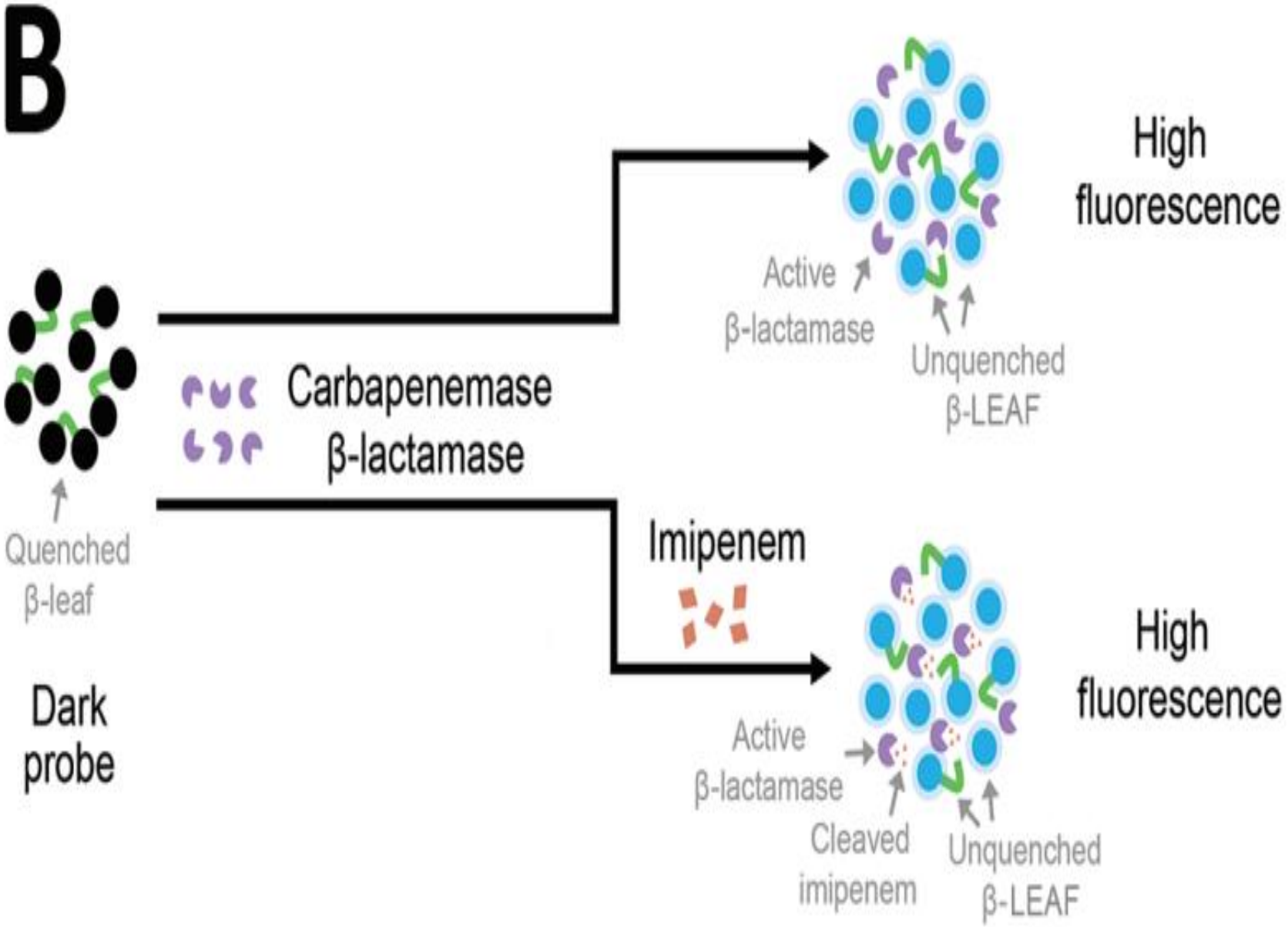
FIBA: Fluorescence identification of β -lactamase activity

- Rapid carbapenemase detection assay
- **Imipenem** (IMP) was added to inhibit non carbapenemase β-lactamases

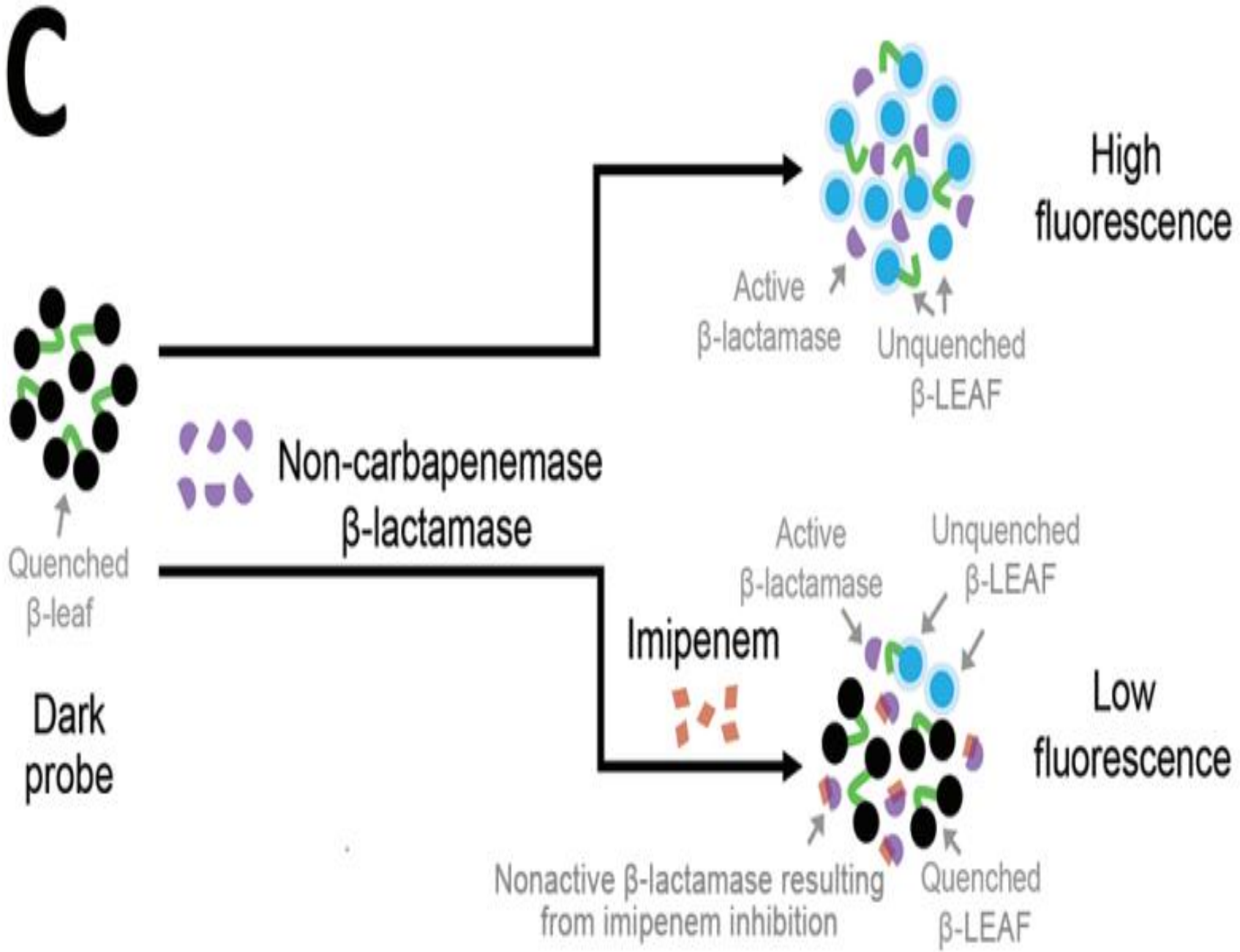


A) The β-lactamase enzyme-activated fluorophore probe

- **This construct was designed to mimic the enzymatic degradation properties**



B) Assay profile for carbapenemase-producing bacteria



C) Assay profile for non–carbapenemase-producing bacteria

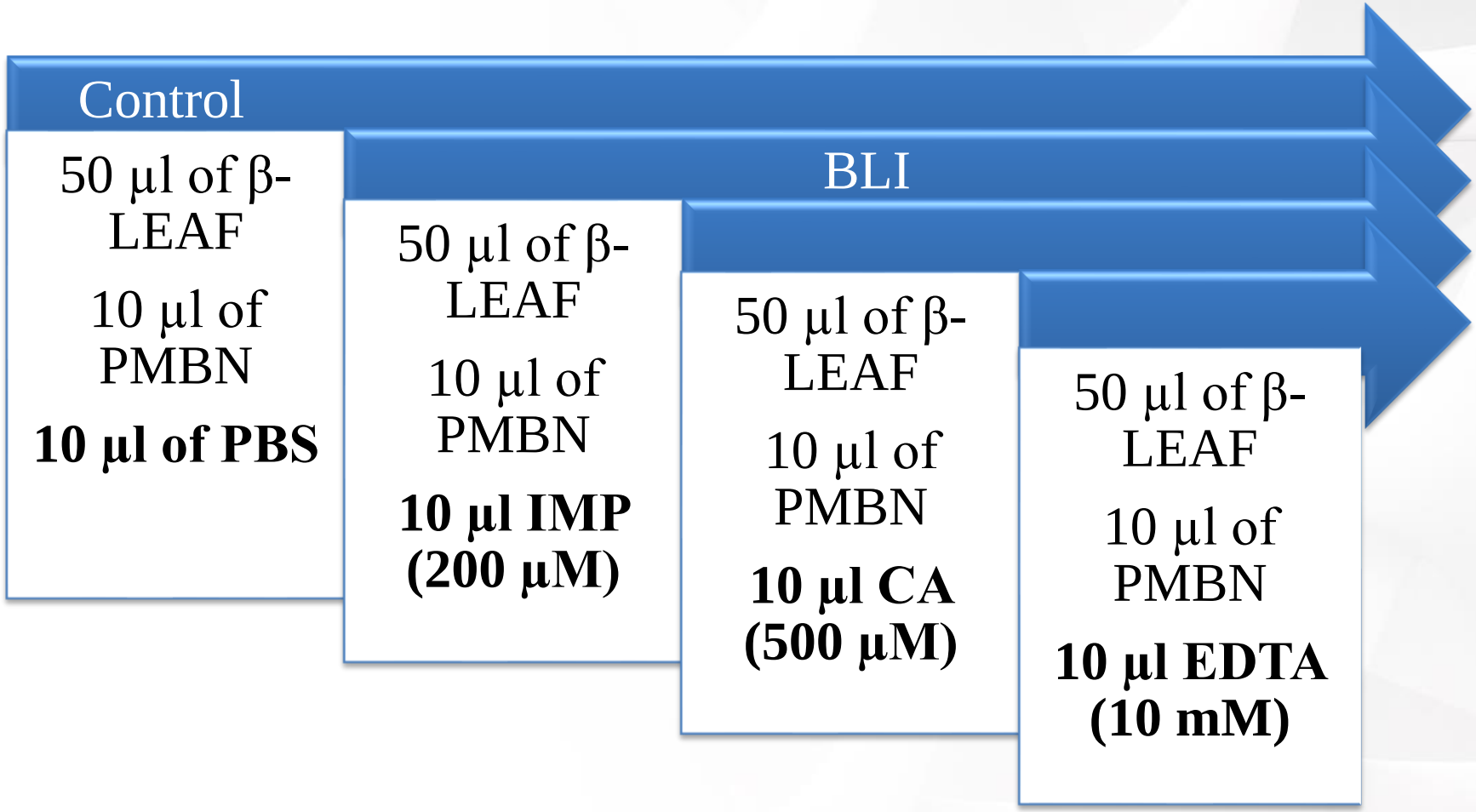
Purpose

- Introducing the carbapenemase type-dependent **BLIs**
- Let's see the FIBA paradigm can be extended beyond simple detection to perform rapid carbapenemase typing with a single mixing step in 10 min?!

MATERIALS & METHODS

- The assay was conducted in a 96-well plate
- Each isolate was tested with a total of 8 wells containing:
  - 50 µl of β-LEAF probe (20 µM)
  - 10 µl of the cell membrane permeabilizer polymyxin B nonapeptide (PMBN, 1 mg/ml)

FIBA assay



Were conducted in duplicate wells

FIBA assay

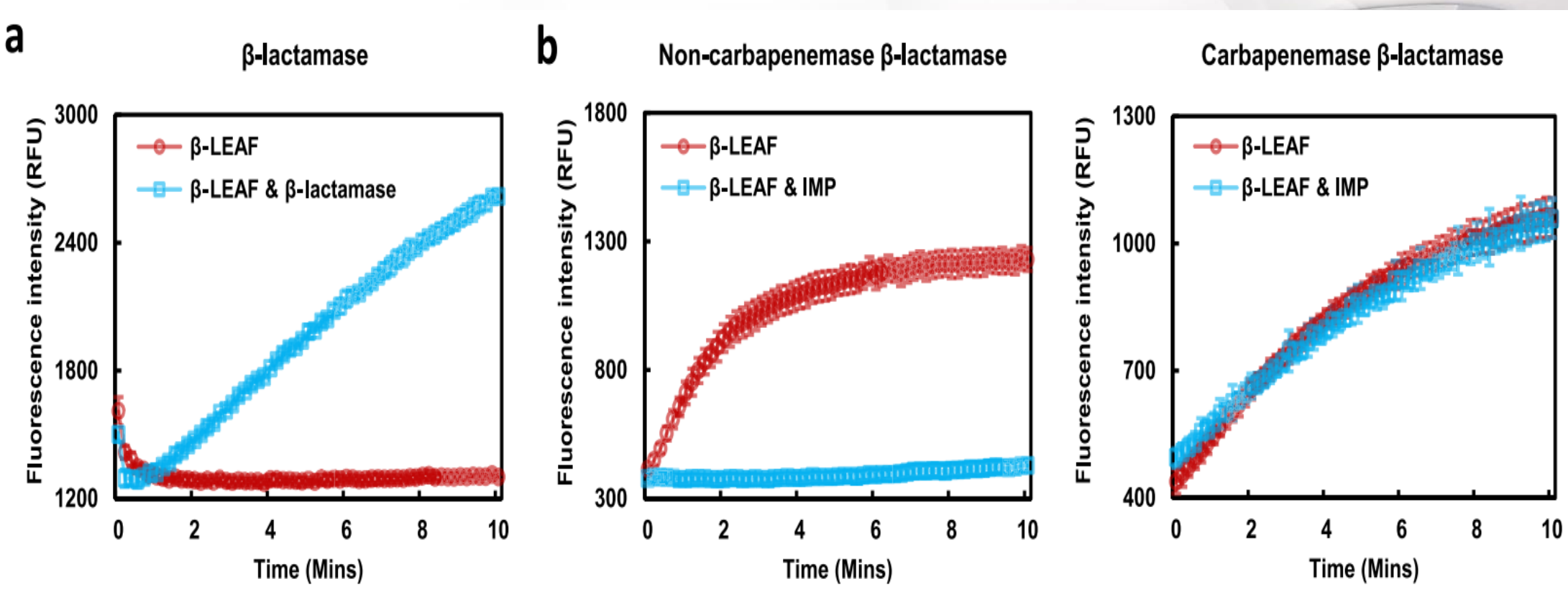
- FIBA with another permeabilizer, **0.1% CHAPS** was performed for β-lactamase **negative**
- This was to rule out **false negatives** due to insufficient permeabilization due to bacterial **polymyxin resistance**
- Before each isolate test, the stored reagents were mixed and aliquoted in 8 wells of a 96-well plate on **ice in the dark**

FIBA assay

- A 30 µl amount of one of the aforementioned bacterial PBS suspensions was added
- Then placed in a fluorescence plate reader and mixed using the plate reader’s shaking function
- The fluorescence increase was monitored by measuring the fluorescence
  - At 37°C
  - At 10-s intervals
  - Excitation/emission at **450/510 nm** for **10 min**

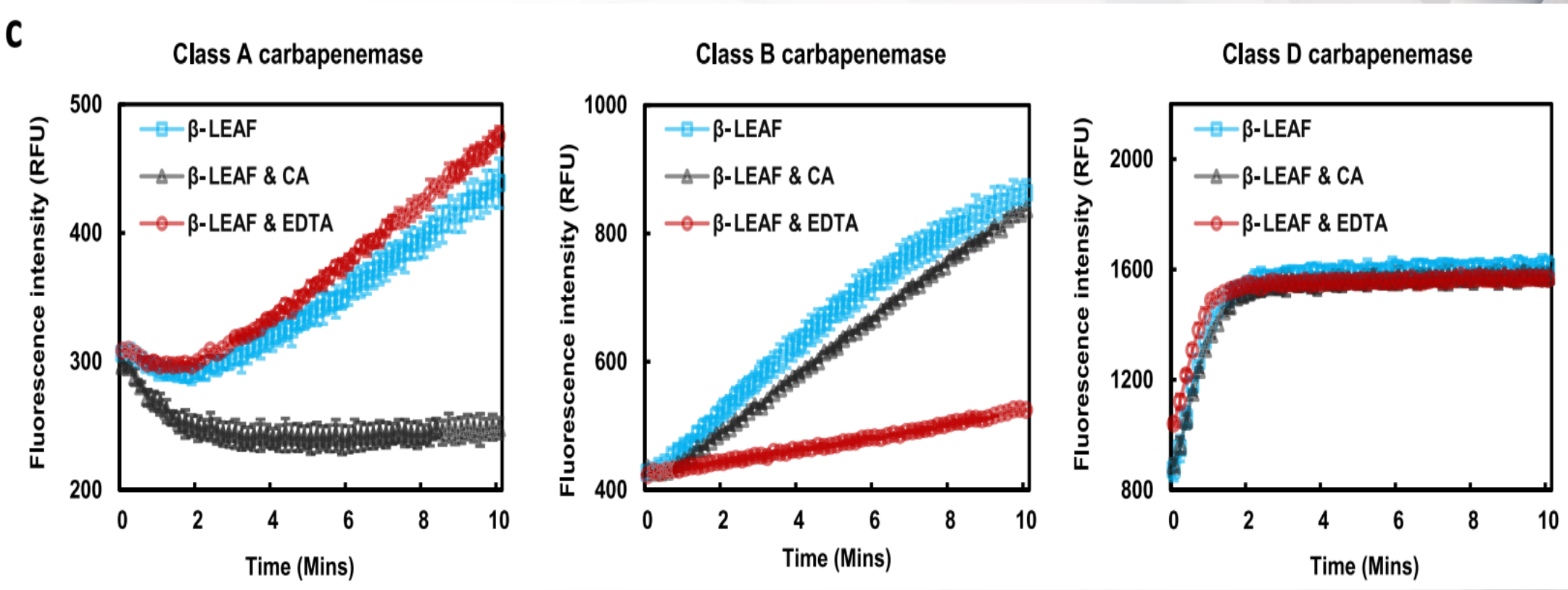
Automated data analysis

- FIBA recognizes carbapenemases and their molecular types by comparing the fluorescence increase **rate (R) of β-LEAF**
  - IMP (non carbapenemase **β-lactamase** inhibitor)
  - CA (class **A** carbapenemase inhibitor)
  - EDTA (class **B** carbapenemase inhibitor)



a) The fluorescence emission behavior by the function of time when strains **producing β-lactamase**

b) carbapenemase and non carbapenemase β -lactamase



c) Different classes of carbapenemases are challenged by β-LEAF alone or β-LEAF together with one of the three inhibitors

Results

- FIBA was tested on **141** human isolates
- The isolates chosen by the Centers for Disease Control (**CDC)** to challenge antibiotic resistance detection assays
- An additional **6** non-carbapenemase-producing isolates were acquired from the American Type Culture Collection (**ATCC**)
- This test panel covers **19 different** bacterial species

Results

- As a reference:
  - Genetic test results for β-lactamases
  - Supplemented with carbapenem susceptibilities
- Samples were physically tested in a **blind** and **random** fashion

Results

- **Among these tested isolates:**
  - 87 isolates are carbapenemase producing
  - 60 isolates are non-carbapenemase producing
- **The isolates without carbapenemases include:**
  - 10 isolates with no β-lactamase
  - 22 with only extended-spectrum β-lactamase (ESBL)
  - 2 isolates with both ESBL & porin modification
  - 18 isolates with only AmpC β-lactamase detected
  - 3 isolates with both ESBL and AmpC β-lactamase
  - 5 isolates with ESBL, porin modification, and AmpC β-lactamase

TABLE 1 Non-carbapenemase-producing isolates subjected to the FIBA test

β-Lactamase type	Species	No. of isolates tested	MIC of[a]: IMP	MRP	ETP	DRP	FIBA test result[b] β-LEAF	+IMP
None	*E. faecium*	1	≤0.5	≤0.12	≤0.12	≤0.12	−	−
	E. cloacae	1	≤0.5	≤0.12	0.5	0.5	−	−
	E. coli	1	≤0.5	≤0.12	≤0.12	≤0.12	−	−
	K. oxytoca	1	≤0.5	1	4	0.5	−	−
	K. pneumoniae[c]	1	2	2	>8	2	−	−
	P. mirabilis[c]	1	8	0.5	0.25	1	−	−
	S. Enteritidis	1	≤0.5	≤0.12	≤0.12	≤0.12	−	−
	S. marcescens[c]	1	≤0.5	≤0.12	≤0.12	≤0.12	−	−
	S. Oslo[c]	1	≤0.5	≤0.12	≤0.12	≤0.12	−	−
	S. Typhimurium	1	≤0.5	≤0.12	≤0.12	≤0.12	−	−
ESBL	*C. koseri*	1	≤0.5	≤0.12	≤0.12	≤0.12	+	−
	E. coli	15	≤0.5	≤0.12–0.25	≤0.12–1	≤0.12–0.25	+	−
	K. oxytoca	1	≤0.5	≤0.12	≤0.12	≤0.12	+	−
	K. pneumoniae[c,d]	6	≤0.5–8	≤0.12–>8	≤0.12–>8	≤0.12–>8	+	−
	S. sonnei	1	≤0.5	≤0.12	≤0.12	≤0.12	+	−
AmpC	*C. freundii*	3	≤0.5–1	≤0.12	≤0.12	≤0.12	+	−
	E. cloacae[d]	4	≤0.5–4	≤0.12–8	0.25–>8	≤0.12–4	+	−
	E. cloacae	1	≤0.5	≤0.12	≤0.12	≤0.12	+	−
	E. coli[d]	4	≤0.5–32	≤0.12–>8	≤0.12–>8	≤0.12–8	+	−
	K. aerogenes[c]	1	≤0.5	≤0.12	1	≤0.12	+	−
	P. aeruginosa	2	16–64	≥ 8	>8	>8	+	−
	S. aureus[c]	3	≤0.5	≤0.12	≤0.12	≤0.12	+	−
ESBL and AmpC	*E. cloacae*	1	≤0.5	≤0.12	1	≤0.12	+	−
	E. coli[d]	5	≤0.5–64	≤0.12–>8	≤0.12–>8	≤0.12–>8	+	−
	K. pneumoniae[d]	2	4–16	1–8	>8	1–8	+	−

TABLE 2 Carbapenemase-producing isolates subjected to the FIBA test

Carbapenemase category			No. of isolates tested	MIC of[a]:				FIBA test result[b]			
Ambler class	Type	Species		IMP	MRP	ETP	DRP	β-LEAF	+IMP	+CA	+EDTA
Class A	KPC	*E. cloacae*[c]	4	2–8	2–8	≥8	2–>8	+	+	−	+
		E. coli	4	2–8	0.5–8	1–>8	0.5–8	+	+	−	+
		E. coli	1	16	8	>8	8	+	−	−	+
		K. ascorbata	1	4	8	8	4	+	+	−	+
		K. oxytoca	1	4	2	2	2	+	+	−	+
		K. pneumoniae[c,d]	33	8–>64	>8	>8	4–>8	+	+	−	+
		M. morganii[c]	1	8	4	8	4	+	+	−	+
		P. aeruginosa	1	>64	>8	>8	>8	+	+	−	+
		P. mirabilis[c]	1	16	2	3	4	+	+	−	+
		S. marcescens[c]	1	>64	>8	>8	>8	+	+	−	+
	SME	*S. marcescens*[c]	8	>32	>8	>8	>8	+	+	−	+
	NMC-A	*E. cloacae*	2	≥32	>8	>8	>8	+	+	−	+
Class B	NDM	*E. cloacae*	1	16	>8	>8	>8	+	+	+	−
		E. coli	3	16–64	>8	>8	>8	+	+	+	−
		P. mirabilis[c]	1	32	4	4	>8	+	+	+	−
	VIM	*P. aeruginosa*[c]	4	4–>64	4–>8	4–>8	4–>8	+	+	+	−
	IMP	*P. aeruginosa*	1	>64	>8	>8	>8	+	+	+	−
Class D	OXA	*A. baumannii*[c]	14	1–>64	0.5–>8	1–>8	0.5–>8	+	+	+	+
		C. freundii	1	4	4	8	2	+	+	+	+
		E. coli	1	>64	>8	>8	>8	+	+	+	+
		K. aerogenes	1	4	2	2	2	+	+	+	+
OXA-48 ←		*K. pneumoniae*[c]	1	8	>8	>8	>8	+	+	−	+
		K. pneumoniae[c]	1	16	>8	>8	8	+	+	+	+

Results

- All but one (*E. coli* with KPC carbapenemase) of the carbapenemase-producing isolates were successfully distinguished

- **Resulting in:**

  - **99% sensitivity** (95% confidence interval [CI], 94% to 100%)
  - **100% specificity** (95% CI, 93% to 100%)

Typing of Carbapenemase

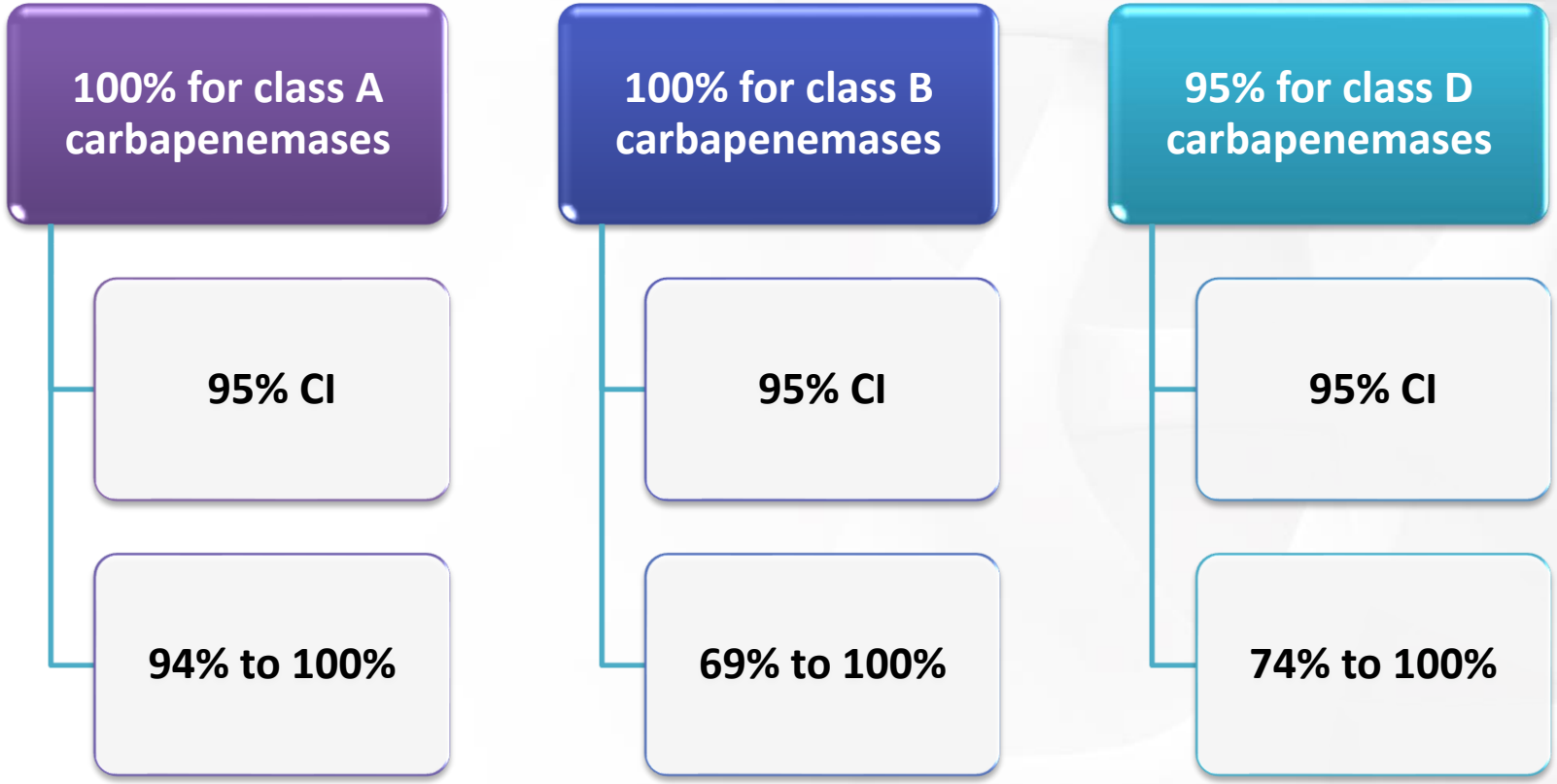
All of the carbapenemase positive isolates were classified **successfully** by FIBA

Except for one class D carbapenemase producer

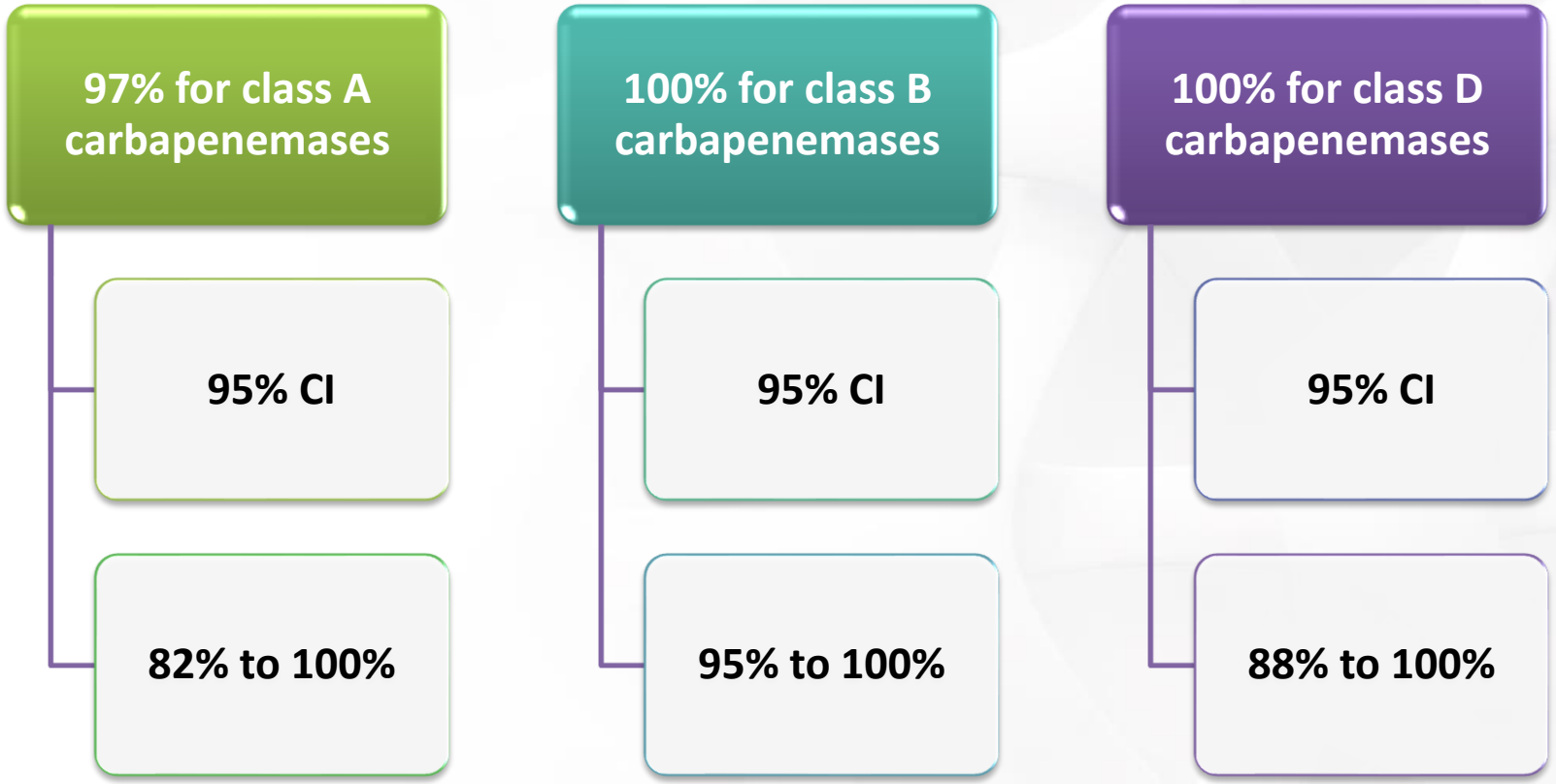
- ✓ *K. pneumoniae* with OXA-48 carbapenemase
- ✓ That was diagnosed as a class A producer

Carbapenemase Classification Sensitivity

This yields a carbapenemase classification:



Carbapenemase Classification Specificity



Discussion

- One carbapenemase-positive isolate was miscategorized as non-carbapenemase producing by FIBA

  - ✓ Probably due to this isolate's remarkably low β-lactamase activity

  - ✓ Which did not allow an efficient hydrolysis of the non-carbapenemase inhibitor, **IMP**

Discussion

- Inhibition of CA, the class A carbapenemase inhibitor in FIBA, was detected in one class D carbapenemase producer

  - ✓ This might be caused by the high concentration of CA in the FIBA assay
  - ✓ Which was applied to overcome the CA resistance in some class A carbapenemases

Discussion

- As a variety of novel BLIs are becoming available
- Potential misclassification of class D carbapenemase would likely be prevented by introducing a specific class D β-lactamase inhibitor in FIBA

Discussion

- Two strains (1 *P. mirabilis* & 1 *P. aeruginosa*) labeled as **β-lactamase negative** with the weak cell permeabilizer PMBN
- Were subsequently found **positive** with the stronger permeabilizer **CHAPS**
  - ✓ As this assay is examined in more-expansive future studies
  - ✓ It will become clearer whether or not the stronger permeabilizer can always be used alone

Discussion

- The only required common laboratory equipment
  - ✓ The fluorescence plate reader, can easily be replaced by portable, low-cost fluorescence readers
- It is close in price (**~$1 per assay**) to the typical phenotypic tests
  - ✓ But significantly **faster** & **less** labor intensive

Discussion

- Compared to **Carba NP**, which is the most rapid test currently used in microbiology laboratories

  ✓ FIBA is more than 10 times faster in carbapenemase identification and typing

  ✓ While maintaining comparable sensitivity and specificity

Discussion

- In terms of future directions, expanded testing of FIBA with more clinical isolates
- Particularly those that are not represented in the current test panel (e.g., IMI and GES)
- Those with lower carbapenem MICs and poorer hydrolytic profiles (e.g., OXA-48, VIM, and SME)

Discussion

- Another essential work for the future is to expand the FIBA paradigm to recognize

  - ✓ The coexistence of carbapenemases from multiple Ambler classes

  - ✓ Since isolates carrying **more than** one molecular class of carbapenemases are emerging

Conclusion

- Finally, as the enhanced detection capabilities of FIBA may open the doors

  - ✓ For simple assays of direct
  - ✓ Uncultured human specimens
  - ✓ Testing on direct specimens is currently in progress

Thank You!!!