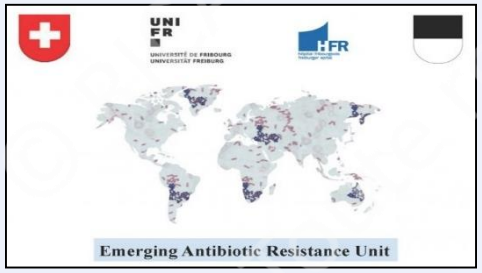
ZHO-1, a novel B1 metallo-beta-lactamases identified from environmental isolates

Nicolas Kieffer*[1], Julia Guzmán Puche[2], Hyo Jung Kang[3], Che Ok Jeon[3], Laurent Poirel[1] Patrice Nordmann[1]

[1]Medical and Molecular Microbiology Unit, Department of Medicine, Faculty of Science, University of Fribourg, Fribourg, Switzerland, [2]University Hospital Reina Sofía-IMIBIC-University of Córdoba, Córdoba, Spain, [3] Department of Life Science and Research Center for Biomolecules and Biosystems, Chung-Ang University, Seoul, 156-756, Republic of Korea

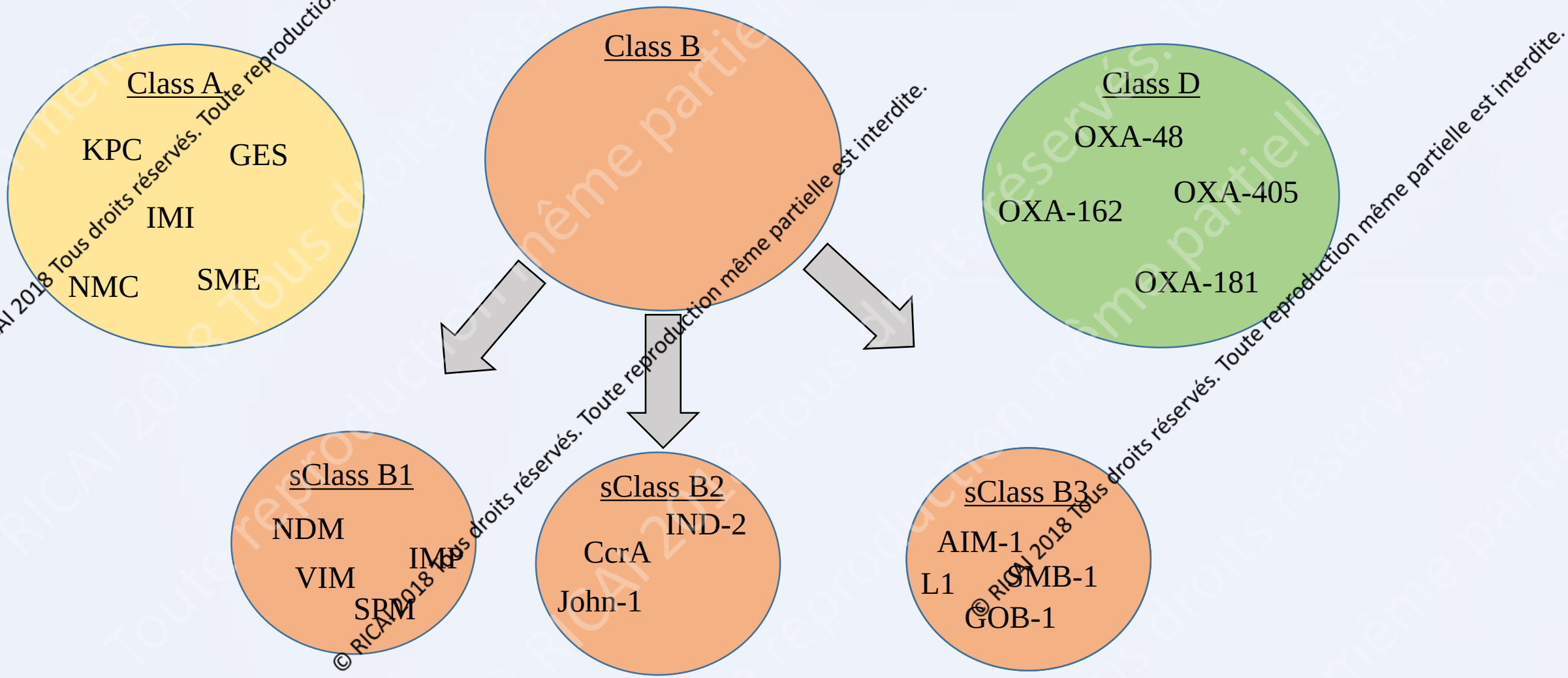


RICAI

NARA
Nationales Referenzlaboratorium zur Früherkennung neuer Antibiotikaresistenzen und Resistenzmechanismen

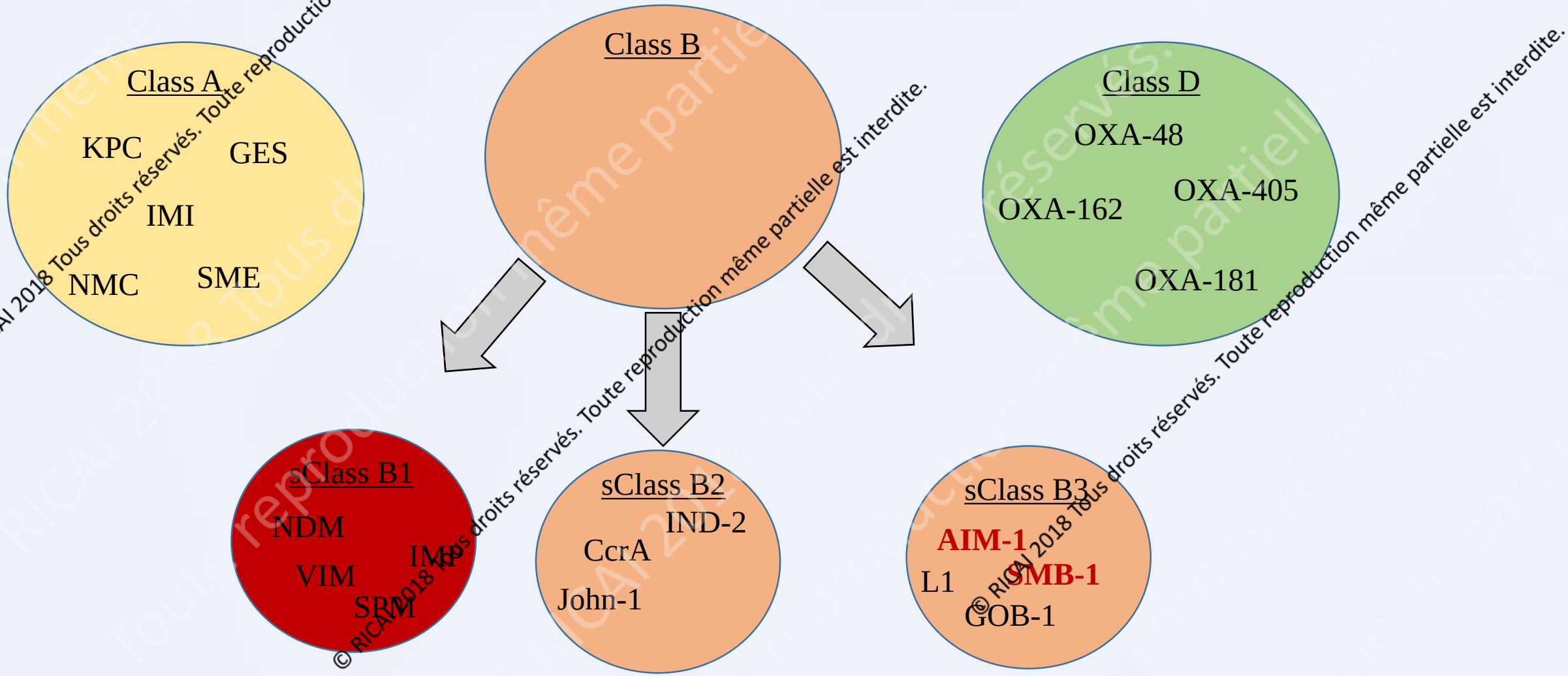
BACKGROUND

- The emergence of carbapenemase-producing bacteria is a major clinical concern



BACKGROUND

- The emergence of carbapenemase-producing bacteria is a major clinical concern



BACKGROUND

- Progenitors of carbapenemases : environmental isolates

  - $bla_{\text{OXA-48 like}}$: *Shewanella* spp.

    → $bla_{\text{OXA-48}}$: *Shewanella oneidensis*

    → $bla_{\text{OXA-181}}$: *Shewanella xiamenensis*

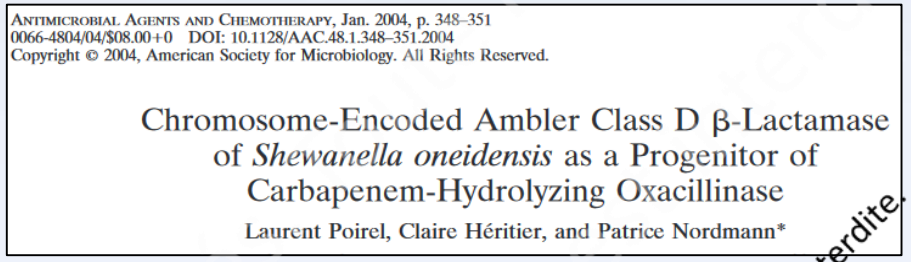
  - $bla_{\text{OXA-23}}$: *Acinetobacter radioresistens*

  → $bla_{\text{NDM-1}}$
  → $bla_{\text{VIM-1}}$
  → $bla_{\text{IMP-1}}$
  → $bla_{\text{SPM-1}}$
  → bla_{KPC}

?

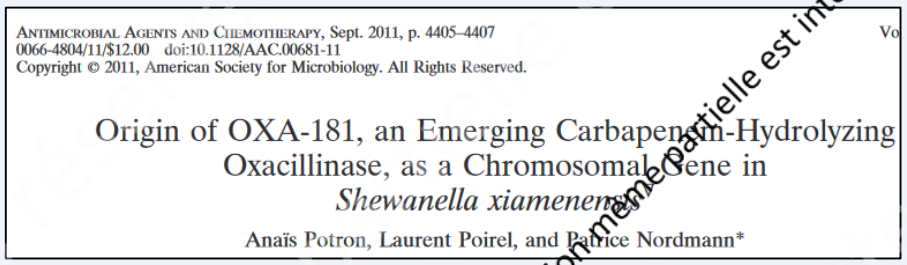
In sillico analysis (blast NCBI)

Progenitors of carbapenemase?


ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2004, p. 348–351
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.1.348–351.2004

Chromosome-Encoded Ambler Class D β-Lactamase of *Shewanella oneidensis* as a Progenitor of Carbapenem-Hydrolyzing Oxacillinase

Laurent Poirel, Claire Héritier, and Patrice Nordmann*


ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2011, p. 4405–4407
0066-4804/11/$12.00 doi:10.1128/AAC.00681-11

Origin of OXA-181, an Emerging Carbapenem-Hydrolyzing Oxacillinase, as a Chromosomal Gene in *Shewanella xiamenensis*

Anaïs Potron, Laurent Poirel, and Patrice Nordmann*

BACKGROUND

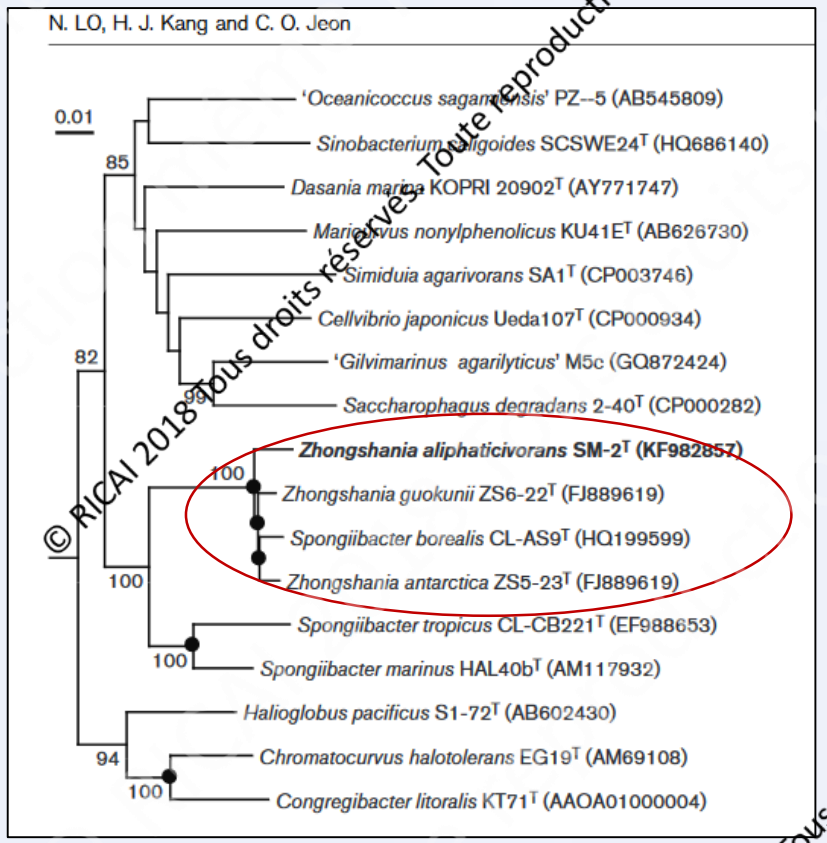
In sillico analysis (blast NCBI excluding all clinical species)

One putative candidate identified

	Max score	Total score	Query cover	E value	Ident	Accession
DIM/SIM/IMP family subclass B1 metallo-beta-lactamase [Zhongshania aliphaticivorans]	504	504	78%	2e-179	100%	WP_082793651.1
metallo-beta-lactamase domain-containing protein [gamma proteobacterium BDW918]	499	499	78%	2e-177	99%	EIF44595.1
DIM/SIM/IMP family subclass B1 metallo-beta-lactamase [gamma proteobacterium BDW918]	490	490	77%	4e-174	98%	WP_086005637.1
subclass B1 metallo-beta-lactamase [Zhongshania aliphaticivorans]	476	476	73%	2e-168	100%	EMO67908.1
PST family subclass B1 metallo-beta-lactamase [Pseudomonas sp. TTU2014-096BSC]	293	293	77%	5e-96	58%	WP_058073988.1
subclass B1 metallo-beta-lactamase PST-1 [Pseudomonas stutzeri]	291	291	77%	2e-95	58%	WP_043942497.1

Presentation of *Zhongshania aliphaticivorans*

- *Z. aliphaticivorans*: new species identified in 2013 from low sea tides sediments on the west coast of South Korea.

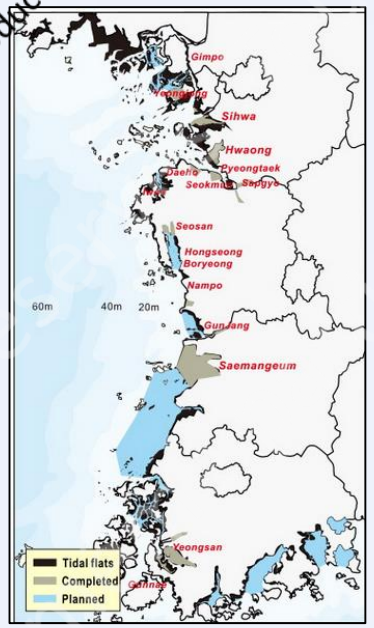


- Gram negative rod
- Halophile (grows on Marine Agar)
- Facultatively anaerobic, motile
- Aliphatic hydrocarbon-consuming bacteria : putative pollutant-degrading bacteria

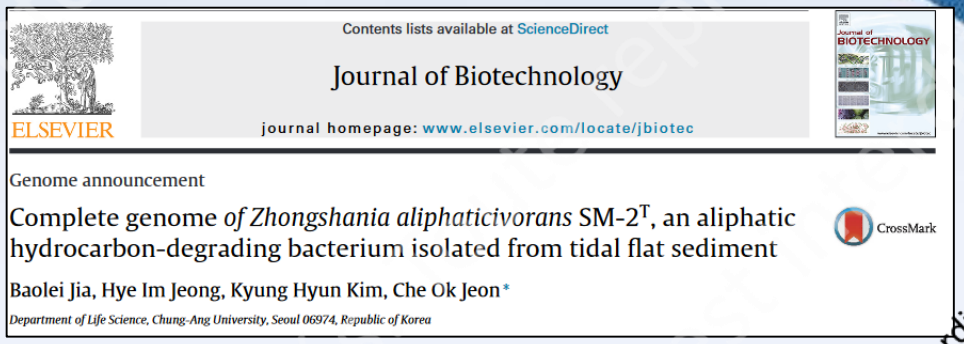
Zhongshania aliphaticivorans sp. nov., an aliphatic hydrocarbon-degrading bacterium isolated from marine sediment, and transfer of *Spongiibacter borealis* Jang *et al.* 2011 to the genus *Zhongshania* as *Zhongshania borealis* comb. nov.

Naysim LO, Hyo Jung Kang and Che Ok Jeon

Department of Life Science and Research Center for Biomolecules and Biosystems, Chung-Ang University, Seoul, 156-756, Republic of Korea



2- Presentation of *Zhongshania aliphaticivorans*


Contents lists available at ScienceDirect

Journal of Biotechnology

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

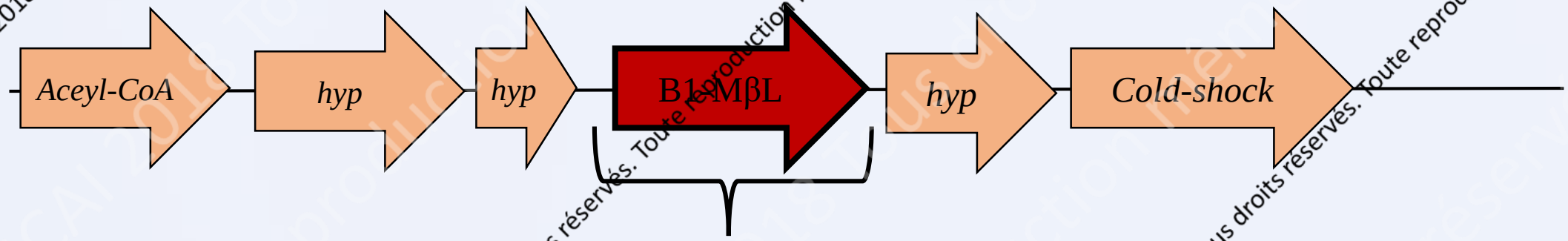
Genome announcement

Complete genome *of Zhongshania aliphaticivorans* SM-2^T, an aliphatic hydrocarbon-degrading bacterium isolated from tidal flat sediment

Baolei Jia, Hye Im Jeong, Kyung Hyun Kim, Che Ok Jeon*

Department of Life Science, Chung-Ang University, Seoul 06974, Republic of Korea

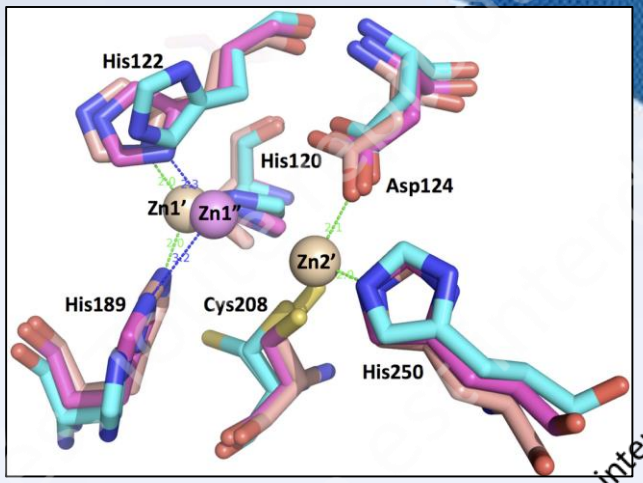
- Genome published recently (2016)
- No mobile element associated with the MβL gene either



***bla*$_{\mathbf{ZHO-1}}$**

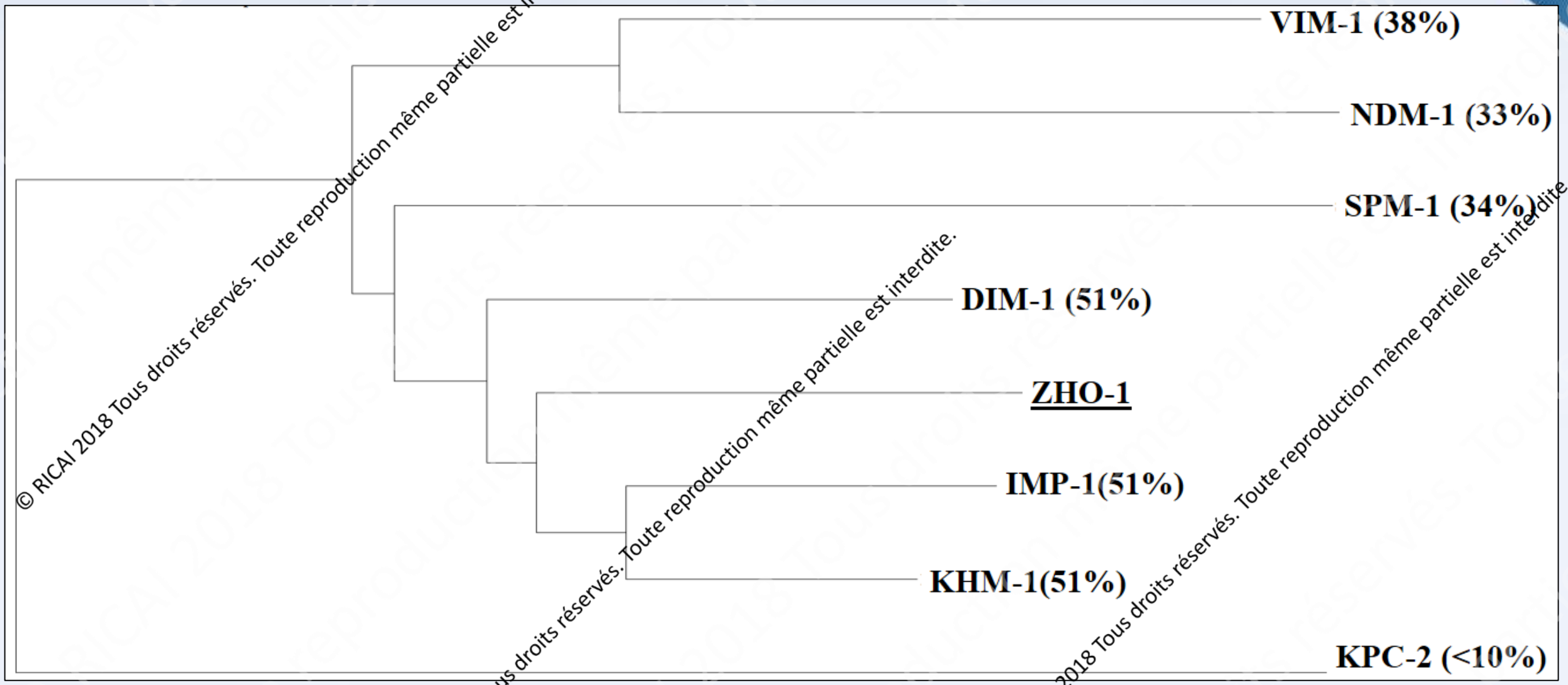
ZHO-1 metallo-β-lactamase

- 51% amino acid identity with DIM-1 IMP-1 and KHM-1, respectively
- Protein of 246 amino acids

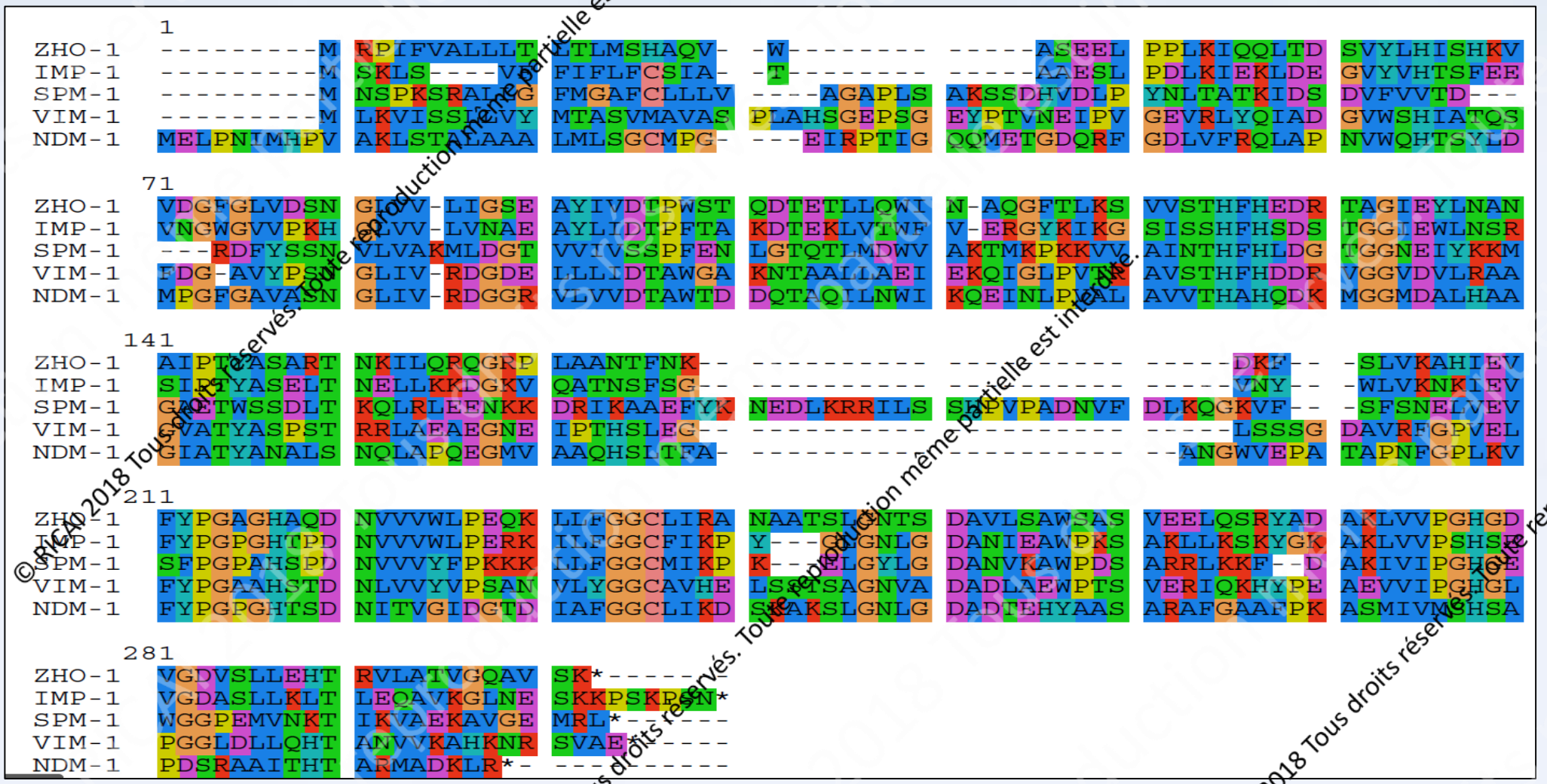


Kim et al, 2011

ZHO-1 metallo-β-lactamase

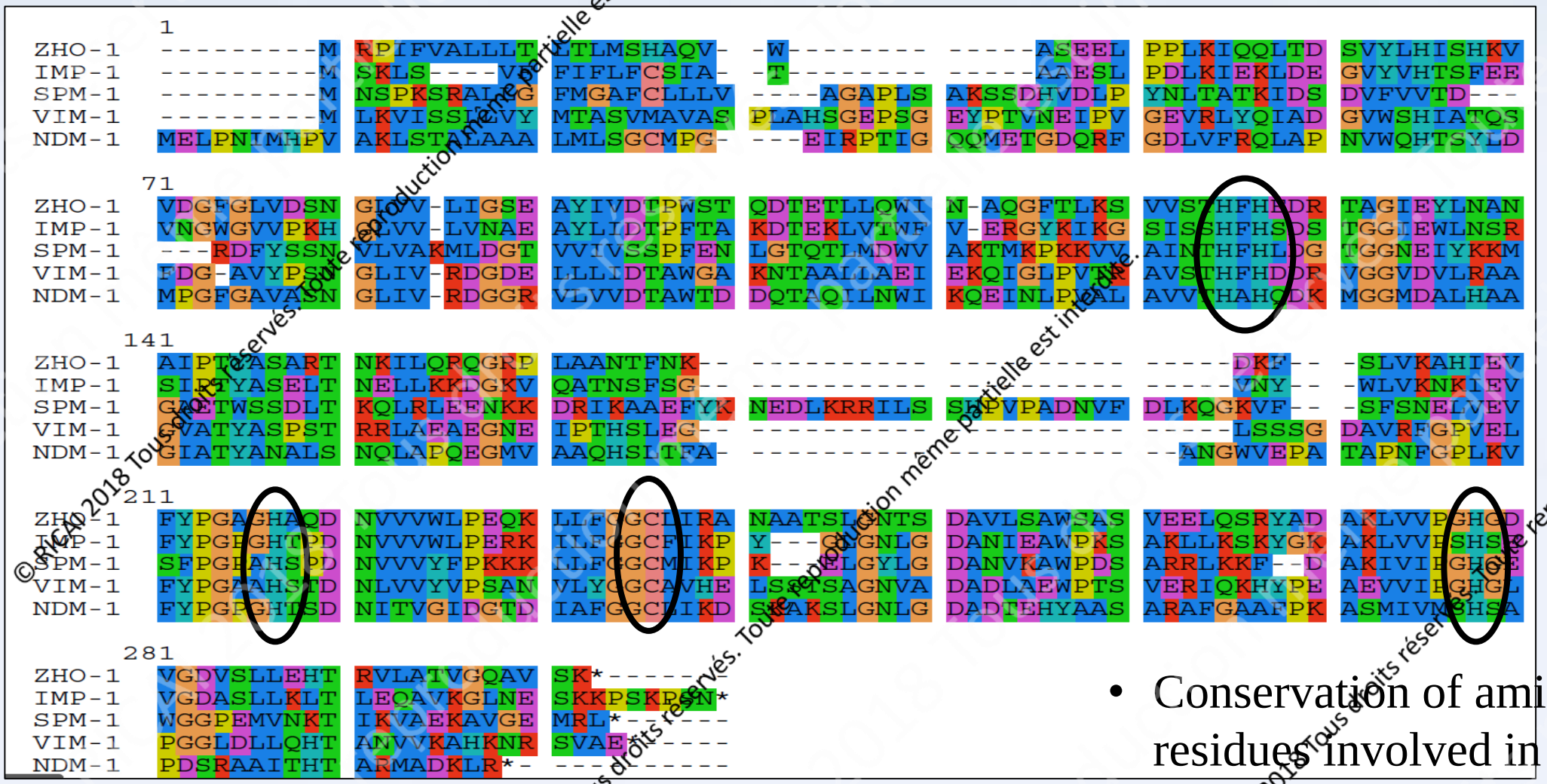


ZHO-1 metallo-β-lactamase



© RICAI 2018 Tous droits réservés. Toute reproduction même partielle est interdite.
© RICAI 2018 Tous droits réservés. Toute reproduction même partielle est interdite.
© RICAI 2018 Tous droits réservés. Toute reproduction même partielle est interdite.

ZHO-1 metallo-β-lactamase



- Conservation of amino acid residues involved in the zinc ions binding:

→ **H_{120}, H_{122}, H_{189}, C_{208}, H_{250}**

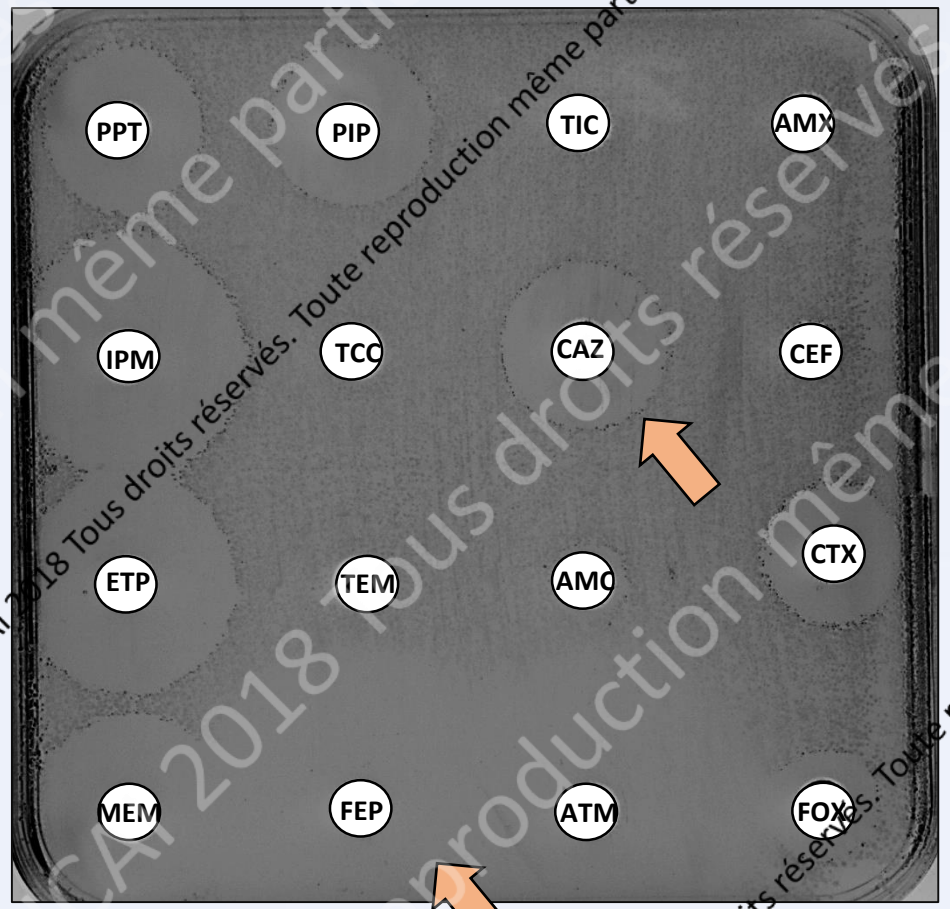
Z. aliphaticivorans susceptibility testing

Isolate susceptible to all beta-lactams

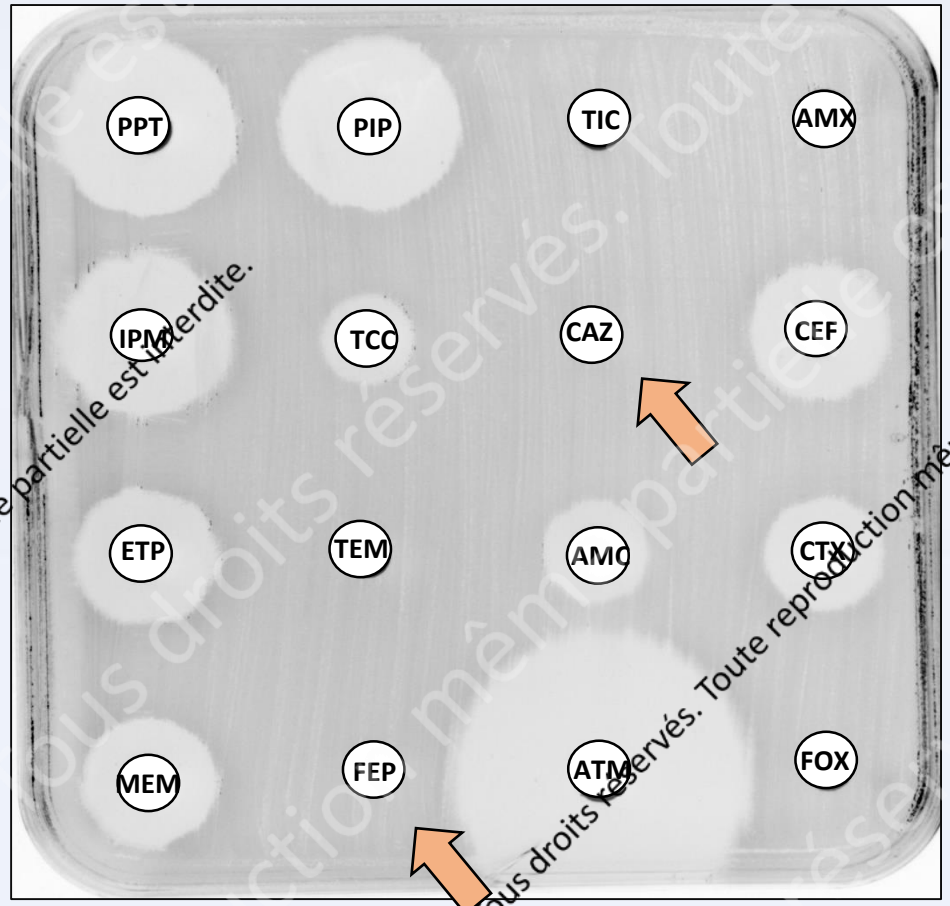
© RICAI 2018 Tous droits réservés. Toute reproduction même partielle est interdite.
© RICAI 2018 Tous droits réservés. Toute reproduction même partielle est interdite.

Resistance phenotype of *E. coli* expressing and ZHO-1

→Comparison between ZHO-1 and IMP-1-expressing clones



***bla*$_{ZHO-1}$ *E. coli* TOP-10**

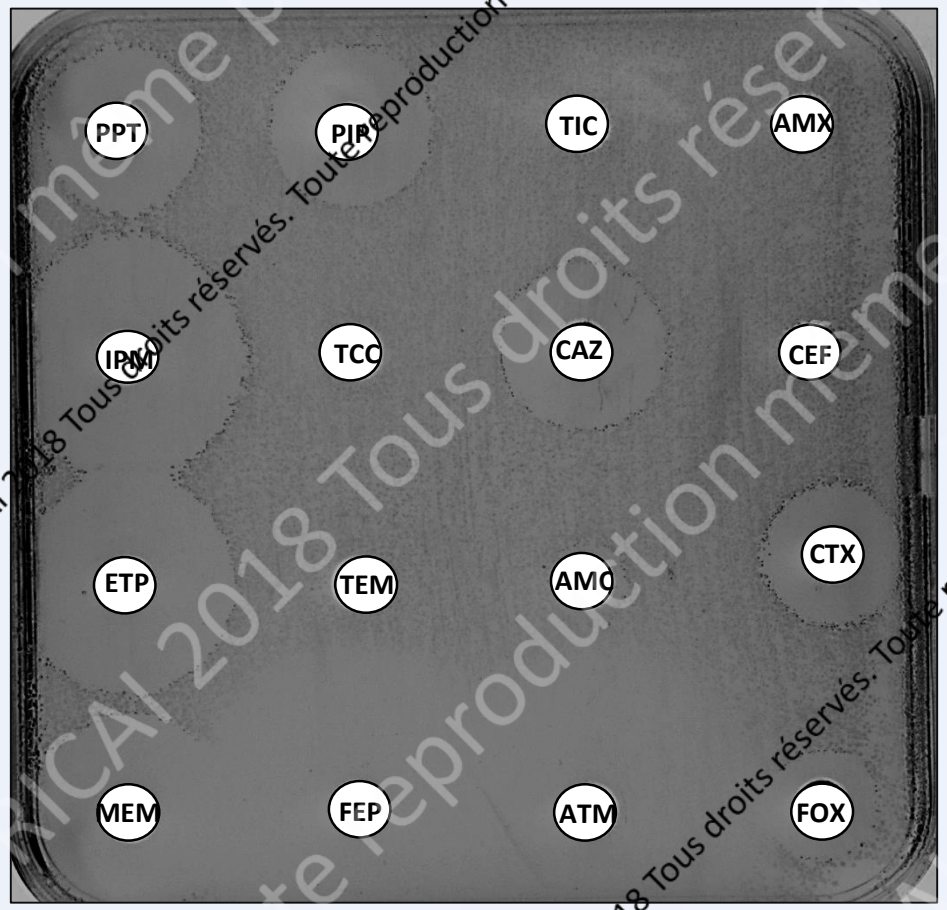


***bla*$_{IMP-1}$ in *E. coli* TOP-10**

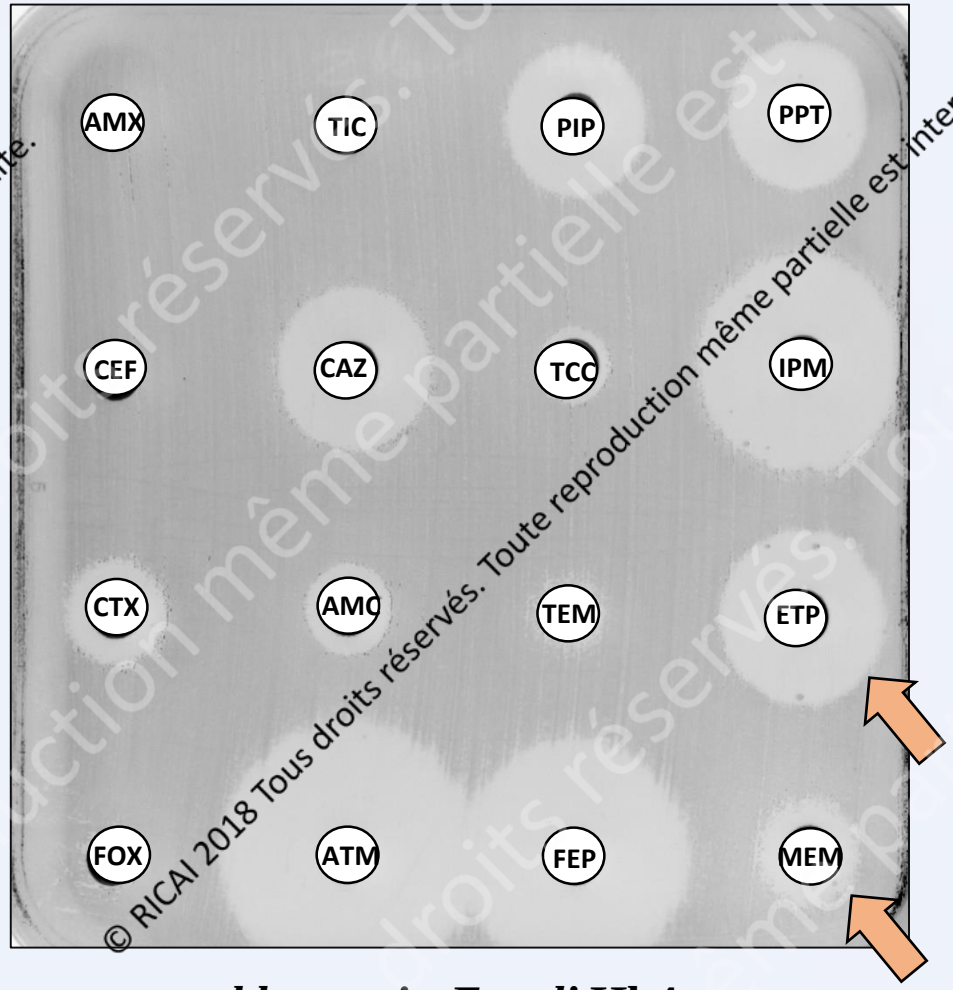
Expression ZHO-1 in porin deficient *E. coli*

Cloning strategy : pTOPO cloning of ZHO-1 in *E. coli* Hb4

***E. coli* Hb4 :** OmpC/OmpF porin-deficient *E. coli*



***bla*$_{ZHO-1}$ *E. coli* TOP-10**



***bla*$_{ZHO-1}$ in *E. coli* Hb4**

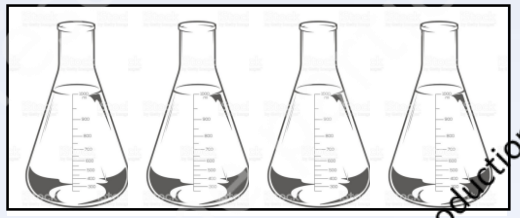
Enzyme purification:

Purification of the enzyme using ÄKTA-prime (GE Healthcare Life Sciences)

- ZHO-1 enzyme:
  - isolectric point: 6,4
  - 246 aa
  - 26.72 kDa

➔ Purification using Q sepharose column with Tris-HCl pH 8,2 and Tris HCl pH 8,2 ; 1M NaCl

Enzyme purifications:

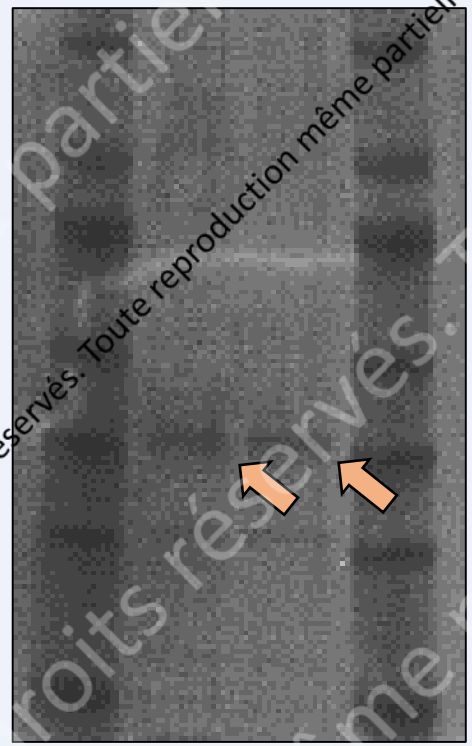


4L of culture

Sonication

ÄKTAPrime system

Nitrocefin assay



SDS-PAGE 12%

Enzymatic activity of ZHO-1

Enzymatic activity using Hepes buffer supplemented with $ZnSO_4$ (50µM)

Antibiotics	k_{cat}(s^{-1})	K_m(µM)	k_{cat}/K_m ($mM^{-1}.s^{-1}$)
Penicillin G	83.6	146.6	570.3
Piperacillin	43	1358	31.6
Cephalotin	4411	19	>10000
Cefoxitin	1.6	147	143
Ceftazidime	0.62	150	4.1
Cefotaxime	25.4	339	749.3
Cefepime	0.26	843	0.3
Aztreonam	<0.01	>1000	ND
Imipenem	39.3	121	324.7
Meropenem	8.1	15.5	519
Ertapenem	ND	ND	ND

Summary:

- We identified 1 new class B carbapenemases from environmental isolates from the sea

- This carbapenemase confers resistance to many β-lactams including carbapenems

- Remaining questions:
  - → Why this metallo-beta-lactamase genes are present in environmental isolates ?
  - → Metallo-β-lactamases in marine environment ?

- Other species carries putative B1-MβL :
  - -*Cellvibrio* sp.
  - -*Gilvimarinus agarilyticus*

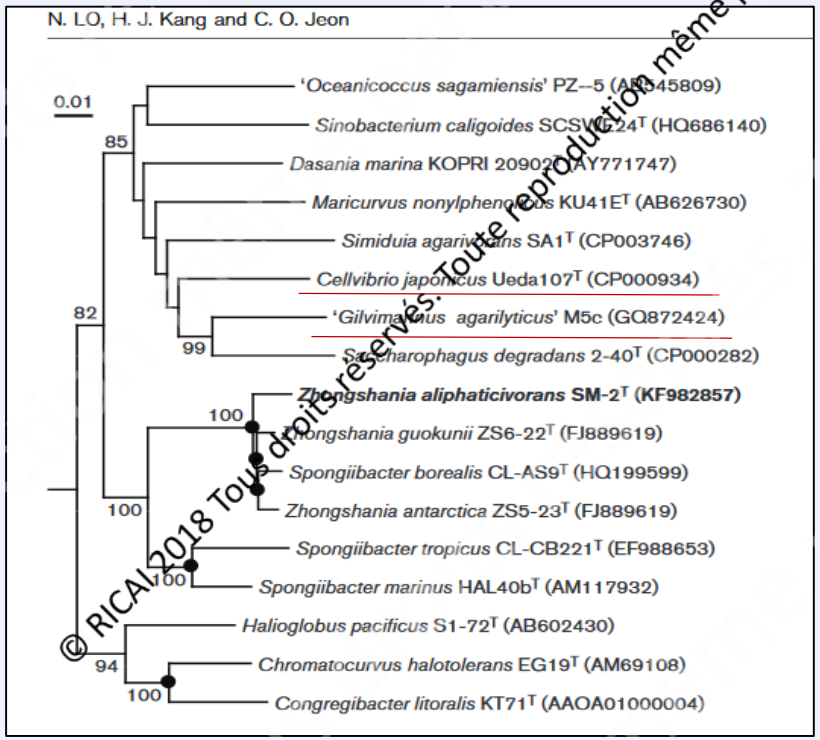


Table 1. MICs of β-lactams for *E. coli* porin proficient and deficient strains with and without the ZHO-1 ß-lactamase gene

MICs (µg/ml)					
Antibiotics	*E. coli* TOP10 (pZHO-1)	*E. coli* HB4* (pZHO-1)	*E. coli* TOP10 empty vector	*E. coli* HB4 empty vector	*Z. aliphacitivorans*
Amoxicillin	**128**	**>128**	2	16	0.5
Amoxicillin+ clavulanic acid[a]	**128**	>128	1	16	0.5
Piperacillin	32	64	1	8	0.5
Cefalotin	**128**	**>128**	4	**128**	0.5
Cefoxitin	**128**	**>128**	2	64	0.5
Ceftazidime	**4**	**128**	0.12	1	0.06
Cefotaxime	**64**	**64**	0.06	0.5	0.06
Cefepime	0.5	4	0.12	0.5	0.06
Aztreonam	0.06	0.5	0.06	0.5	0.06
Imipenem	0.5	0.5	0.06	0.06	0.06
Meropenem	1	**4**	0.06	0.25	0.06
Ertapenem	1	**4**	0.06	1	0.06

Grey shaded MICs values correspond to intemediate susceptibility; boldened MIC values correspond to resistance.

[a]Clavulanic acid was added at a fixed concentration of 2 µg/ml. **E. coli* HB4 is an OmpC/OmpF porin-deficient strain.