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Evaluation of a novel selective medium, CHROMagar™ Acinetobacter with KPC supplement, for detection of multidrug-resistant *Acinetobacter baumannii* from clinical specimens in Japan

Sayoko KAWAKAMI[1)], Miwa ASAHARA [2)], Naohisa MATSUNAGA[1)4)], Yasuo ONO[3)], Taiji HURUKAWA[2)], Ryouichi KUBO[5)], Masanobu ONOZAKI[5)], Tomota NAKANO[5)], Takamasa KANEKO[5)], Tamio TERAMOTO[4)], Yukihisa MIYAZAWA[1)]

Department of Infection Control and Prevention, Teikyo University Hospital[1)], Department of Central Laboratory, Teikyo University Hospital[2)], Department of Microbiology and Immunology, Teikyo University School of Medicine[3)], Department of Medicine, Teikyo University School of Medicine[4)], Kanto Chemical Co., Inc.[5)]

2-11-1 Kaga Itabashi-ku Tokyo 173-8606 Japan Tel. +81-3-3964-1211(7793) Fax. +81-3-3964-9327

Objectives

Multidrug-resistant *A. baumannii* (MDRAB) has recently been reported in both western countries and in China. However, cases of such infections are very rare in Japan.

Here, we report hospital-acquired infection by *A. baumannii* bla_{OXA-66} resistant to the carbapenems imipenem(IMP) or meropenem(MEPM), the aminoglycoside amikacin(AMK) and the fluoroquinolones levofloxacin(LVFX) or ciprofloxacin(CPFX).

We also evaluated the novel chromogenic medium, CHROMagar™ Acinetobacter (CHROMagar, France) supplemented with KPC to detect MDRAB.

Methods

- KPC-supplemented CHROMagar™ Acinetobacter was used for isolation of drug-resistant strains, such as MDRAB bla_{OXA-66}, MDRAB $bla_{OXA-66 + OXA-23}$, *Acinetobacter bla* $_{IMP-1}$, multidrug-resistant *P. aeruginosa* (MDRP) bla_{IMP-1}, MDRP bla_{VIM-2}, permeability decreasing MDRP, *P. putida*, *S. maltophilia*, *E. coli* $bla_{CTX-M-2}$, *K. pneumoniae* bla_{KPC} and *E. cloacae* bla_{IMP-1}. There were incubated at 35℃ for 18 – 72 h.
- For the clinical trial, 5,740 specimens from the pharyngeal swabs, urine and rectal swabs, and 6,617 swab specimens from environmental materials were plated on this medium and incubated at 35℃ for 18 – 72 h.

Agar□

Acinetobacter□ KPC supplement

supplement□

1. Heating at 100 degrees to dissolve the agar powder
2. Cool to 60 degrees
3. Added to the medium *Acinetobacter*□ supplement and KPC supplemet□
4. Dispense 20ml of each petri dish

Test of strains □

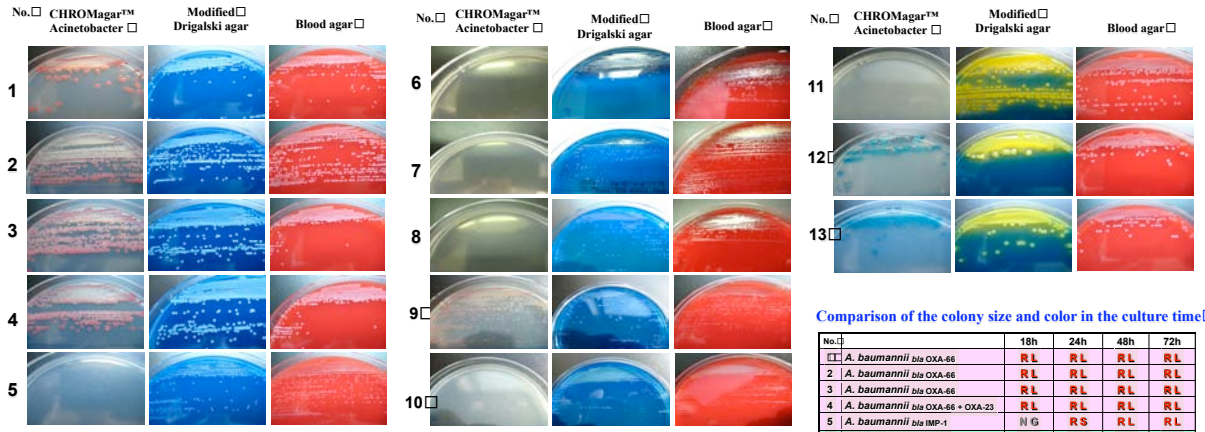
No.□		IPM	MEPM	AMK	LVFX	CPFX
□	*A. baumannii* bla_{OXA-66}	>32	>32	>256	>32	>32
2	*A. baumannii* bla_{OXA-66}	1	2	4	8	4
3	*A. baumannii* bla_{OXA-66}	4	8	>256	4	>32
4	*A. baumannii* $bla_{OXA-66 + OXA-23}$	>32	>32	32	>32	>32
5	*A. baumannii* bla_{IMP-1}	>32	>32	4	0.125	0.125
6	*P. aeruginosa* bla_{IMP-1}	>32	>32	8	2	2
7	*P. aeruginosa* bla_{VIM-2}	>32	>32	>256	>32	>32
8	*P. aeruginosa* OMP	>32	>32	>256	16	16
9	*P. putida*	0.5	2	4	0.5	0.5
10	*S. maltophilia*	>32	>32	32	0.5	0.5
11	*E. coli* $bla_{CTX-M-2}$	0.25	0.064	4	>32	>32
12	*K. pneumoniae* bla_{KPC}	>32	>32	64	>32	>32
13	*E. cloacae* bla_{IMP-1}	>32	>32	4	4	4

Results 1

- In the trials of stock strains, three genotypes of MDRAB showed red and large colonies after cultivation for 18 h at 35℃.
- MDRP bla_{VIM-2} did not grow on the medium, permeability decreasing MDRP showed small red colonies after 24 h of cultivation and MDRP bla_{IMP-1} yielded red small colonies after 48 h of cultivation.
- ESBL-producing enteric bacilli did not grow on the medium. However, *K. pneumoniae bla* $_{KPC}$ and *E. cloacae* bla_{IPM-1} grew as small blue-green colonies after 18 h of cultivation.

Results 1

Comparison of color and size of colonies after culturing for 18 hours at 35 ℃



Comparison of the colony size and color in the culture time□

No.□		18h	24h	48h	72h
□	*A. baumannii* bla_{OXA-66}	R L	R L	R L	R L
2	*A. baumannii* bla_{OXA-66}	R L	R L	R L	R L
3	*A. baumannii* bla_{OXA-66}	R L	R L	R L	R L
4	*A. baumannii* $bla_{OXA-66 + OXA-23}$	R L	R L	R L	R L
5	*A. baumannii* bla_{IMP-1}	N G	R S	R L	R L
6	*P. aeruginosa* bla_{IMP-1}	N G	N G	R M	R M
7	*P. aeruginosa* bla_{VIM-2}	N G	N G	N G	N G
8	*P. aeruginosa* OMP	N G	R S	R M	R M
9	*P. putida*	R S	R S	R M	R M
10	*S. maltophilia*	N G	R S	R M	R M
11	*E. coli* $bla_{CTX-M-2}$	N G	N G	N G	N G
12	*K. pneumoniae* bla_{KPC}	B S	B M	B L	B L
13	*E. cloacae* bla_{IMP-1}	B S	B M	B L	B L

R L: red large, R M:red medium, R S: red small,
B L:blue large, B M:blue medium, B S:blue small, N G:no growth□

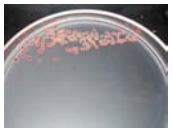
Results 2

- Twenty-one MDRAB were detected from clinical and environmental specimens.
- Clinical and environmental isolates with carbapenems MIC ≥ 2 mg/ml (*P. aeruginosa*, *P. fluorescens*, *S. maltophilia*, *C. indologenes* and *A. xylosoxidans*) grew as small red colonies on this medium after cultivation for 24 – 48 h.
- *P. putida* isolates with carbapenems MIC < 1 mg/ml yielded small red colonies on this medium after incubation for 18 h.
- Among red colonies suspected *Acinfetobacter* were easily discriminated from other genus by oxidase test and gram staining.

A case of isolated MDRAB from stool□

Modified Drigalski agar

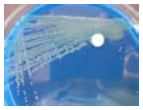
E. coli is predominant, MDRAB not separated



CHROMagar™ Acinetobacter □

MDRAB selection was excellent

A case of isolated MDRAB from lavatory□



Too many species can not be identified MDRAB

MDRAB selection was excellent

Clinical test results□

Inspected	Pharyngeal swabs, urine , rectal swabs	Environmental specimens
Number of tests	5740□	6617□
MDRAB positive	8□	13□

Conclusion

- The novel selective medium CHROMagar™ Acinetobacter supplemented with KPC was useful for detecting our cases with MDRAB bla_{OXA-66} MDRAB $bla_{OXA-66+OXA-23}$ in Japan.
- In addition, it was especially valuable for active surveillance of specimens containing multiple bacteria, such as those from the pharynx, urine, faeces and the environment.