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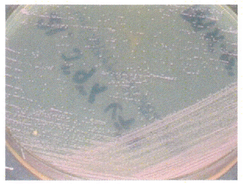
Evaluation of new CHROMagar MRSA to detection of MRSA from the Clinical Specimens

Y.Otsuka[1,3],T.Tsubata[1], M.Onozaki[2], R.Kubo[2], H.Hanaki[3]

[1]Social Insurance Central General Hospital, Tokyo, Japan, [2]Kanto Chemical Co.,Inc.,Tokyo,Japan,[3]Kitasato Research Center for Anti-infection Drugs, Tokyo,Japan

Fig.2: Aspect of MRSA colony on Each Selective Plate

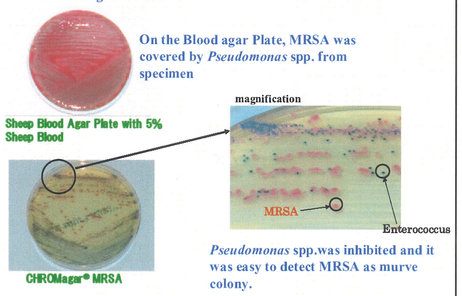


MRSA on CHROMagar® plate

MRSA on OPA Agar plate

Incubation for 24hr at 35℃

Fig. 3:Comparing the colony of MRSA on Sheep Blood Agar and CHROMagar®MRSA



On the Blood agar Plate, MRSA was covered by *Pseudomonas* spp. from specimen

Pseudomonas spp.was inhibited and it was easy to detect MRSA as murve colony.

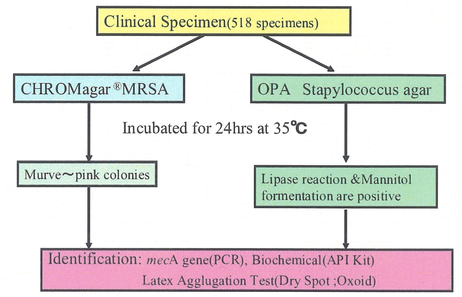
(Incubated for 24 hours at 35℃)

Table.1: Comparion of Productivity of each selective media by Miles&Misra technique

Media	Incubation Time	Tenfold Dilutions & Growth level on Each plate						Diameter of typical colonies (mm)
		10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	
Sheep Blood Agar TSAII with 5% Sheep Blood-1	18h	3+	2+	1+	1+	1+	1+	1
	24h	3+	3+	2+	1+	1+	1+	2
	48h	3+	3+	2+	1+	1+	1+	3
Sheep Blood Agar TSAII with 5% Sheep Blood-2	18h	3+	2+	1+	1+	1+	0	1
	24h	3+	3+	2+	1+	1+	0	2
	48h	3+	3+	2+	1+	1+	0	3
OPAStaph Agar-1	18h	3+(−)	2+ (−)	1+ (−)	0	0	0	0.1
	24h	3+(±)	3+(±)	2+ (−)	1+(−)	0	0	0.5
	48h	3+(+)	3+(+)	2+(+)	1+(+)	1+(−)	0	1.5
OPAStaph Agar-2	18h	3+(−)	2+ (−)	1+ (−)	0	0	0	0.2
	24h	3+(−)	3+(±)	2+(−)	1+(−)	0	0	0.5
	48h	3+(+)	3+(+)	2+(+)	1+(−)	0	0	1.5
CHROMagar MRSA-1	18h	3+	2+	1+	1+	0	0	0.5
	24h	3+	3+	2+	1+	0	0	1
	48h	3+	3+	2+	1+	0	0	2
CHROMagar MRSA-2	18h	3+	2+	1+	1+	0	0	0.5
	24h	3+	3+	2+	1+	0	0	1
	48h	3+	3+	2+	1+	0	0	2.5

*(+):lipase reaction positive,(−) lipase reaction negative,(±)very weak reaction was appeared

Fig.1: Evaluation Method for Each Plate (Recovery Test)



Introduction

Low level resistant MRSA has often caused infection in Japan. The MRSA has mecA gene and is induced to become high level resistant by antibiotic-chemotherapy. Thus it is sometimes difficult to detect the MRSA using traditional MRSA Screen Media and sometimes missed it in routine tests.

Recently CHROMagar MRSA was launched as novel chromogenic media which can detect this MRSA.

We studied how many low level MRSA can be recover clinical specimens comparing the CHROMagar MRSA with traditional Screen Media.

Materials and Method

Microorganism: The 518 specimens including sputa and pus were collected after starting antibiotic chemotherapy between April 2004 and January 2005 from patients at Social Insurance Central General Hospital. The specimens were inoculated on CHROMagar MRSA(CHROMagar,France) and OPA Staphylococcus Agar plates (BBL Ltd.)and incubated for 24hrs aerobically. Growth of colonies showing pink or mauve coloration on CHROMagar plates was considered MRSA, and colonies showing mannitol fermentation and lipase reaction(Egg Yolk reaction) on OPA plates were considered as MRSA.The presumptive MRSA colonies from those two-plate media were identified *mec* A gene by PCR.(Fig.1)

The productivity ratio of those two media was determined by Miles & Misra technique; that is , MRSA type strain(ATCC 43300) was multiple diluted by saline, and each dilution dropped on TSAII Sheep Blood Agar(BD Ltd.), CHROMagar MRSA, and OPA Staphylococcus Agar gradually.

Result

Number of presumptive isolates from the 518 specimens for each selective plate were137 strains of CHROMagar and 85 strains of OPAstreptococcus Agar. 39 of MRSA strains were lipase reaction negative, and it was difficult to detect the MRSA by OPA Staphylococcus Agar. (Fig.2) *mec* A gene was detected out of all presumptive isolates on CHROMagar MRSA by PCR, and it was fadmitted 16strains were detected from CHROMagar MRSA and 3 strains, OPA Staphylococcus Agar.The growth result by Miles & Misra test is shown in the Table.1. In this study, we could not able to recognize significant difference of number of the colony between CHROMagar MRSA and OPA Staphylococcus Agar, but typical colony sizes of MRSA on CHROMagar MRSA was significantly larger than on OPA plate. When incubation for 24hrs, though it was difficult for MRSA to recognize the lipase reaction on OPA Staphylococcus Agar Plate was not clear, and while MRSA colony on CHROMagar MRSA were colored clearly in marve.

Discussion

CHROMagar MRSA is designed selective media to induce and detect low level MRSA and contains antibiotics of cephamycin system in stead of Oxacillin in traditional MRSA screen media.

In this study, we recognized that CHROMagar MRSA showed significant improvement in number of recovery of MRSA, and growth on the CHROMagar plate was also superior to traditional one.

In Clinical specimens, *Pseudomonas aeruginosa* is grown on the traditional MRSA screen plate,and they often covered and hid the colony of MRSA (Fig.3) and missed them. In this study, MRSA from 41 of 137 specimens were detected by CHROMagar MRSA, and it was approved to be difficult to recover MRSA on traditional plate because interference of the *Pseudomonas* spp.

In addition, the contrast between the color of colonies and the background color on CHROMagar MRSA were clearer than traditional ones.

However, when the specimens were spread thickly, CHROMagar sometimes allowed to grow non-MRSA microorganisms including MSSA.

Conclusion

Novel chromgenic media CHROMagar MRSA is easier to detect MRSA than traditional Screen media which is based on lipase reaction and it may be useful to screen MRSA from clinical specimens.

We expect the CHROMagar MRSA support to control nosocomial infection as screening media.

Contact Information
FAX : +81-3-3364-5663
E-mail : bac@gaea.ocn.ne.jp