2013 EDITION

Selection of Publications

chromID™ by bioMérieux

MULTIDRUG-RESISTANT ORGANISM SCREENING and *CLOSTRIDIUM DIFFICILE* CULTURE

BIOMÉRIEUX

chromID® Selection of Publications – 2013 Edition

Since the first edition of the chromID® Selection of Publications in 2007, followed by an update in 2010, both the status of knowledge and diagnostic testing performance in the field of multidrug-resistant organisms (MDROs) have evolved significantly. This 2013 edition therefore provides summaries of the most recent studies and proof of performance of the bioMérieux chromID® range for MDRO screening and *Clostridium difficile* culture.

Chromogenic media offer enhanced bacterial identification over traditional culture media through colour differentiation. The intensity and specificity of the colours of chromID®* media optimise microbial enumeration, making it easy to rapidly identify bacterial colonies.

Our range of chromogenic media for screening MDROs covers:

- methicillin-resistant *Staphylococcus aureus* (MRSA),
- carbapenemase-producing *Enterobacteriaceae* (CPE),
- extended spectrum β-lactamase producing *Enterobacteriaceae* (ESBL),
- *Enterococcus faecalis* and *E. faecium* with acquired Vancomycin resistance (VRE),

and combines detection of resistance mechanisms and identification of bacteria.

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The chromID® range specifically responds to the rise in healthcare-associated infections and the increasing need for multidrug-resistant bacterial screening in hospitals today.

➔ 2013
chromID® MRSA SMART**
chromID® CARBA SMART** (bi-plate)
chromID® ESBL / chromID® VRE (bi-plate)
chromID® OXA-48

➔ 2012
chromID® CARBA
chromID® MRSA / chromID® S. aureus (bi-plate)

➔ 2011
chromID® C.difficile

➔ 2007
chromID® ESBL
chromID® VRE

➔ 2005
chromID® MRSA

* chromID® chromogenic media are not available in all countries.
Contact your local representative for more information on availability in your country.
** Under development.

chromID® MRSA

chromID® MRSA agar* is a chromogenic medium for the screening of methicillin-resistant *Staphylococcus aureus* (MRSA) in chronic carriers or patients who are at risk for MRSA.

MRSA are multi-resistant bacteria which may cause healthcare-associated infections. The detection of MRSA carriers is particularly important for the prevention and monitoring of these infections. In this context, the use of chromID® MRSA agar contributes towards the active surveillance of MRSA.

chromID® MRSA agar consists of a rich nutrient base combining different peptones. It also contains a chromogenic substrate of α-glucosidase and a combination of several antibiotics, which favour:
- the growth of methicillin-resistant staphylococci (MRSA) including hetero-resistant strains.
- the direct detection of MRSA strains by revealing α-glucosidase activity (patent registered).

The selective mixture inhibits most bacteria not belonging to the genus *Staphylococcus*, as well as yeasts.

* See package insert for more information

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JOURNAL OF CLINICAL MICROBIOLOGY
2013;51:318-319

Detection of oxacillin-susceptible *mec*A-positive *Staphylococcus aureus* isolates by use of chromogenic medium MRSA ID

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Reported cases of oxacillin-susceptible (OS) *mec*A-positive *Staphylococcus aureus* strains are on the rise. Due to their susceptibility to oxacillin and cefoxitin, it is very difficult to detect them by using routine phenotypic methods. This article describes two such isolates that were detected by chromogenic medium and confirmed by characterization of the *mec*A gene element.

This is the first study to report the usefulness of a chromogenic medium for the detection of OS-MRSA. Results show that *S. aureus* isolates that are positive on MRSA ID plates but negative by cefoxitin screening and susceptible to oxacillin should be investigated further for the presence of PBP2a by latex agglutination or by PCR for the *mec*A gene.

The use of a chromogenic medium in combination with latex agglutination is a simple and effective method to detect OS-MRSA, which will otherwise be classified as methicillin-susceptible *S. aureus* because of its susceptibility to oxacillin and cefoxitin.

KEY POINTS

- → chromID® MRSA has the capability to detect the increasingly encountered profile of oxacillin-susceptible MRSA with the presence of *mec*A gene.
- → Detection of OS-MRSA allows better antibiotic stewardship, as treatment of such patients with ß-lactams may cause an increase in oxacillin MICs, ultimately leading to failure of therapy.

EUROPEAN JOURNAL OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES
2012;331:1765-70

Culture-based detection of methicillin-resistant *Staphylococcus aureus* by a network of European laboratories: an external quality assessment study

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This study aimed to: (i) assess the performance of laboratories in European hospitals in detecting methicillin-resistant *Staphylococcus aureus* (MRSA) using culture-based methods, and (ii) obtain an overview of the prevalence and epidemiology of MRSA, as well as current screening practices used at the participating hospitals.

Twenty-three hospital laboratories in eleven European countries and Israel participated in this external quality assessment (EQA) of the culture-based detection of MRSA. Participants also reported the MRSA prevalence in clinical cultures and patient screening specimens, and their current MRSA screening.

An EQA panel of 18 samples consisting of two MRSA harbouring SCCmec IV and I, and one strain each of methicillin-resistant coagulase-negative *S. epidermidis*, methicillin-sensitive *S. aureus* and *Escherichia coli* as pure strains or in mixtures from 10^7 to 1 cfu absolute loads was analysed by the 23 participants. Seventeen (74%) participants identified 17 or more samples correctly. Of these, 15 (88%) utilised a chromogenic medium alone (ChromID, bioMérieux; BBL CHROMagar, BD Diagnostics; MRSA Select, Bio-Rad Laboratories) or combined with a conventional medium and up to three confirmatory tests.

This major EQA found that most laboratories used rapid chromogenic media as in-house protocols for MRSA detection. MRSA prevalence in clinical cultures varied widely among participating hospitals, even within the same country (ranging from 11–20% to 61–70%), in comparison to MRSA carriage rates, which were quite similar across the different countries (0-20%). Most participants combined screening of nares with other sites (45%), as well as screening ICU patients alone, or with other high-risk patient groups (68%).

KEY POINTS

- → In this major Quality Assessment study, chromID® MRSA is the chromogenic medium most widely used in 23 European hospital laboratories.
- → chromID® MRSA also shows the best sensitivity.

JOURNAL OF CLINICAL MICROBIOLOGY
2012;50:2506-8

Detection of methicillin-resistant *Staphylococcus aureus* in clinical specimens from cystic fibrosis patients by use of chromogenic selective agar

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(d) Microbiology Department, Universidade Federal de Ciências da Saúde de Porto Alegre, Porto Alegre, Brazil

This study evaluated the use of a specific and selective chromogenic medium (MRSA ID, commercialized as chromID® MRSA) to detect methicillin-resistant *Staphylococcus aureus* (MRSA) in cystic fibrosis (CF) respiratory clinical samples.

A total of 138 *S. aureus* isolates were detected from a total of 179 consecutive clinical specimens (128 sputa and 51 oropharynx swabs) recovered from 130 CF patients. Of these, 54/138 were mecA positive. Fifty-four MRSA isolates were detected by MRSA ID, while only 24/54 (44%) (odds ratio [OR], 2.79; 95% confidence interval [CI], 1.63 to 4.76) were detected by conventional methods.

TABLE 1: Evaluation of conventional and surveillance cultures for recovering *S. aureus* isolates

		No. of isolates that grew on each culture type/total no. of isolates (%)	
PCR result(n)	Read time (h)	Conventional	Surveillance*
mecA-positive (54)	24	16/54 (29.6)	44/54 (81.5)
	48	24/54 (44.4)	54/54 (100)
mecA-negative (84)	24	63/84 (75)	2/6 (NA)
	48	84/84 (100)	6/6 (NA)

* NA: not applied

This is the first study evaluating the use of a chromogenic medium containing cefoxitin for surveillance in CF patients. Based on the study results, the authors recommend the incorporation of a chromogenic selective medium, such as MRSA ID, to enhance the recovery of MRSA from CF respiratory specimens and improve MRSA surveillance in CF patients.

KEY POINTS

→ The use of a chromogenic medium such as chromID® MRSA enhances the recovery of MRSA in cystic fibrosis patient samples.

JOURNAL OF HOSPITAL INFECTION
2012;81:20-24

Evaluation of chromogenic meticillin-resistance *Staphylococcus aureus* media: sensitivity versus turnaround time

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This study aimed to investigate the effects of incubation time on the performance of commercial chromogenic MRSA media. In a clinical setting, incubation times generally vary between 16 and 48 h.

The study initially compared the early recovery of meticillin-resistant *Staphylococcus aureus* (MRSA) isolates from routine screening swabs following 16-23 h to that after 22-24 h of incubation using ChromID MRSA (bioMérieux). It also compared ChromID MRSA, ChromID MRSA V2 (bioMérieux), Brilliance MRSA 2 Agar (Oxoid) and Colorex MRSA (E&O Laboratories Ltd) as selective media for 6035 MRSA screening swabs.

Only 303 of 623 (48.6%) MRSA isolates detected by this medium after 48 h were recovered after 16-23 h compared with 726 of 1018 (71.3%) isolates after 22-24 h. After implementing the 22-24 h incubation period, 50 (4.4%) isolates exhibiting a positive chromogenic reaction gave conflicting coagulase latex and DNase results. In 88% of these cases, DNase gave the correct result of non-MRSA. ChromID MRSA demonstrated the highest sensitivity overall at 93.2%, followed by Colorex MRSA Agar (87.1%), ChromID V2 (83.7%) and Brilliance Agar (78.2%). All media exhibited specificities of >99.7%.

In this study, we found that extending an initial overnight incubation period for chromID MRSA from 16-23 to 22-24 h significantly improved the "early" detection of MRSA from 48.6% to 71.3% of total MRSA isolates recovered after 48 h. ChromID MRSA was more sensitive after its full recommended incubation time of 48 h than the most sensitive '24 h medium' (Colorex MRSA).

The optimal choice of chromogenic medium for MRSA screening should be determined according to the priority of the user. If maximum sensitivity is the priority, ChromID MRSA is appropriate; however, if a 24 h turnaround is deemed a higher priority, then Colorex MRSA Agar would be a more appropriate choice. Confirmatory tests in addition to coagulase latex agglutination should be used for the identification of MRSA from ChromID MRSA.

KEY POINTS

➔ The choice of a chromogenic medium is determined by the priority given by the user: chromID® MRSA answers the need for maximum sensitivity.

JOURNAL OF HOSPITAL INFECTION
2009;71:373-388

MRSA screening: throat swabs are better then nose swabs

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The MRSA screening in our hospital has been to routinely take 3 swabs from each patient (nose, throat, perineum) with additional samples taken in specific circumstances (skin lesions) and urine (in catheterized patients). Faced with a huge increase in the number of MRSA screenings, we have considered the possibility to reduce the number of sample sites. However, before proceeding to any such policy, we have performed some studies.

All the samples (635 sets) have been cultured on chromID MRSA, bioMérieux. A set included: throat, nose and perineum specimens.

MRSA was isolated from 119 of these 635 screening set. Throat swab provided the largest overall number of MRSA isolates in patients and also the largest number of MRSA isolates in patients not colonized at other sites. In other words, omitting throat swabs would have resulted in a greater reduction of positive MRSA screens (by 19 %).

Specimen type (number of specimens)	Total MRSA isolates from this site	MRSA isolates only from this site
Throat (635)	72	23
Nose (635)	59	11
Perineum (635)	55	8
Skin lesion swabs (141)	32	7
Catheter urine (80)	8	1

This study demonstrated that multiple anatomic sites are useful to increase the recovery rate of MRSA screening. The importance of throat swab sampling for MRSA screening should be recognized.

KEY POINTS

→ chromID® MRSA is validated with nares, throat, perineum, groin, and skin specimens. By using a combination of sites, sensitivity is improved.

Comparison of PBP2a latex agglutination assay, PBP2a rapid immunochromatographic assay, and chromogenic medium for identification of methicillin-resistant *Staphylococcus aureus* directly from positive blood cultures

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INTRODUCTION

Rapid and accurate detection of methictllin-resistant *Staphylococcus aureus* (MRSA) from clusters of Gram-positive cocci in blood cultures is important for the prompt initiation of effective antimicrobial therapy. Presently, several methods for the direct or rapid detection of MRSA from blood culture specimens are used, including chromogenic agar, direct cefoxitin disk susceptibility testing, penicillin-binding protein 2a (PBP2a) latex agglutination (LA) testing, and various molecular methods. Whereas the first two methods require at least 1 day to generate a complete result from a blood culture, the PBP2a LA test requires an additional day and the use of an isolated colony. In contrast, molecular testing often requires additional expertise and instrumentation that may not be readily available in many clinical laboratories. The EZ~StepMRSA rapid kit (DINONA, Iksan, Korea) is a novel screening test for the direct detection of MRSA in positive blood cultures that uses a lateral-flow chromatography immunoassay (ICA) to detect PBP2a and requires only 5 h to complete after obtaining a positive signal on an automated blood culture system. The aim of this study was to compare the three non-molecular methods, PBP 2a LA, PBP2a rapid kit, and MRSA chromogenic medium (CM), for direct detection of MRSA.

MATERIALS AND METHODS

In total 100 *S. aureus* (50 MRSA and 50 methicillin susceptible *S. aureus* (MSSA) confirmed by *mecA and nue* PCR) were seeded into blood culture bottles (BacT/Alert 3D, USA). When the isolates gave a positive signal, 5-mL blood from culture broth was added to three serum separator tubes and each tube was centrifuged at 1,300 g for 10 min. Two pellets were used as the inocuJum after three washes for direct PBP2a LA (Denka Seiken, Japan) and MRSA-CM (ChromID MRSA, bioMérieux, France). PBP2a LA and MRSA-CM were subsequently tested by manufacturer's instructions. For PBP2a ICA (pBP2a MRSA rapid kit, DiNona. Korea), a pellet was inoculated to BHI broth containing 4 ug/ml, cefoxitin for 4 h at 35°C. Then, 10 min after the addition of 0.1 mL of lysis buffer and Tween 20, the lysate was tested using the MRSA rapid kit. The results were interpreted within 20 min, according to the manufacturer's instructions (Figs. 1 & 2). .

RESULTS

The results in PBP2a LA test could be diversely interpreted as the observers due to the ambiguous agglutinations in both MRSA and MSSA, which were scored as negative in this study. All 11 negative isolates with PBna LA test showed positive using isolated colony, but 7 of 11 negative isolates showed ambiguous results using the pellet directly. Only one isolate among 50 *mec*A positive isolates (MRSA) showed negative results by PBP2a ICA but this isolate changed to positive after an 8-h incubation. The sensitivities and specificities of the tests for the direct detection of MRSA was 78 and 100% for PBP2a LA, 98 and 100% for PBP2a ICA, 100 and 100% for MRSA-CM, respectively (Table 1).

Figure 1. EZ-Step MRSA rapid kit for detection of MRSA from blood culture with.Gram positive cocci in clusters. Lanes 1, 2, MRSA (positive); lane 3, MRCNS (positive); lanes 4,5, MSSA (negative); lanes 6,7, MSCNS (negative)

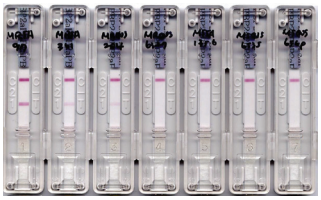

Figure 2. The process chart for 3 direct detection methods of MRSA from positive blood cultures.

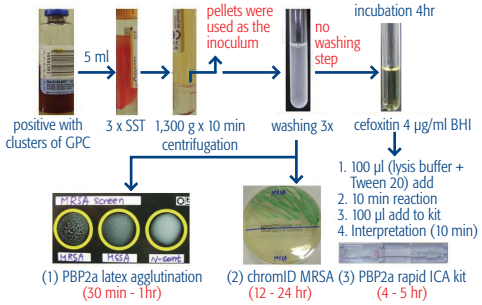

Table 1. The results of 3 direct detection methods of MRSA from positive blood cultures.

nue positive *Staphylococcus aureus* (No.)	PBP2a LA		PBP2a ICA		ChromID-MRSA	
	P	N	P	N	P	N
mecA positive (50)	39	11[a](7[b])	49	1[d]	50	0
mecA negative (50)	0	50(5[b])	0	50	0	50
Sensitivity (%)	78.00 (92[c])		98.00		100	
Specificity (%)	100 (90[c])		100		100	
PPV(%)	100		100		100	
NPV (%)	81.97		98.00		100	

a) PBP2 LA using isolated colony later showed all 11 isolates as positive. b) The ambiguous results produced by PBP2a LA were scored as negative. c) Sensitivity and specificity of PBP2a LA with a positive interpretation of ambiguous agglutination. d) After 8-h incubation, the result changed to the positive.

CONCLUSIONS

MRSA-CM and PBP2a ICA produced a superior result to PBP2a LA. PBP2a ICA was comparable with MRSA-CM in terms of accuracy. MRSA-CM is more effective than PBP2a ICA in terms of the simplicity of the procedures and the clarity of interpretation, but PBP2a ICA takes less time to complete (>12 h) than MRSA-CM. Thus, PBP2a ICA is useful for early management of MRSA bacteremic patients at hospitals lacking the capability to perform molecular testing for MRSA.

KEY POINTS

- → The use of chromID® MRSA on seeded or artificially spiked blood culture is comparable to immunochromatography assays (ICA).
- → chromID® MRSA is easy-to-use and gives same accurate results as ICA.

Novel *mecA* variant LGA251 - ability to detect isolates using commercial methods

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INTRODUCTION

Recently a novel *mecA* variant, *mecA*$_{LGA251}$ was discovered in England, Scotland and Denmark (Garzia-Alverez, Lancet Inf. Dis 2011;11:595-603). *mecA*$_{LGA251}$ has less than 70% homology to the *mecA* gene and only 63% aminoacid similarity to PBP2a. This raises issues about detection and confirmation of methicillin-resistance for such strains.

Here we present the performance of different commercial phenotypic and genotypic systems used in microbiology labs on 72 *mecA*$_{LGA251}$ positive isolates from Denmark and France.

MATERIALS AND METHODS

Isolates: 71 human and 1 animal *mec*A negative, *mecA*$_{LGA251}$ positive and phenotypic MRSA isolates. All isolates were typed by *spa*-typing.

Gold standard: Detection of *mecA*$_{LGA251}$ by PCR

Phenotypic tests:

- **Disk diffusion** using cefoxitin and EUCAST methodology.
- **MIC** for FOX and OXA using BMD, AST-P581 (Vitek, bioMerieux), PMIC/ID-60 (Phoenix, Becton Dickinson), Pos MIC Panel Type 31 (Microscan, Siemens).

Chromogenic MRSA plates: MRSA Select (BioRad), ChromID MRSA (bioMérieux), BBL CHROMagar MRSA II (Becton Dickinson), Brilliance MRSA 2 (Oxoid).

Immunologic tests: Clearview Exact PBP2a (Alere), PBP2a agglutination (Oxoid).

Molecular tests: BD GeneOhm StaphSR assay (Becton Dickinson), GeneXpert MRSA/MSSA SSTI and nasal (Cepheid), NucliSENS EasyQ MRSA (bioMérieux). DNA microarray StaphyType (Alere).

RESULTS

60 isolates belonged to CC130 (9 different *spa* types, all *agr* 3) and 12 to CC1943 (4 different *spa* types, all *agr* 4) (Table 2).

Disk diffusion:

All 72 isolates were detected as MRSA using cefoxitin disk diffusion.

MIC Broth Microdilution:

Oxacillin MICs were distributed between 0.5 mg/L and 16 mg/L. 5 isolates had MICs ≤2 mg/L. Results for cefuroxime, ceftriaxone and meropenem are shown in Table 1.

Automated MIC systems:

Data for the β-lactam antibiotics are shown in Table 2.
Based on cefoxitin and the expert systems of the machines using Microscan, Vitek, and Phoenix, 2, 3 and 12 isolates were misidentified as MSSA, respectively.
Oxacillin gave lower MICs than BMD with all three systems and oxacillin MICs were in the susceptible range for all three systems (Table 1).
All isolates were susceptible to other classes of antibiotics including fluoroquinolones, macrolides, aminoglycosides, tetracyclines, rifampicine, fusidic acid, mupirocin, oxazolodinones and vancomycine.

Other phenotypic methods:

By Clearview Exact PBP2a assay (Alere), 10 isolates were positive using colonies on blood agar plates but all 72 were positive after cefoxitin induction. By agglutination PBP2a test (Oxoid), all were negative except one regardless of cefoxitin induction (Table 4).

By ChromID (bioMérieux) and Brilliance MRSA 2 (Oxoid) none were misidentified whereas by MRSA Select (bioRad) and CHROMagarII (BD) 35 and 12 isolates, respectively were misidentified (Table 2.)

Genotypic methods: All tested isolates (n=20) were negative using GeneOhm, GeneXpert and NucliSENS assay. Using DNA microarrays, no resistance gene was found except *tet* Efflux and *fos*B (n=27). Some isolates harboured toxin and virulence genes: *tst* (n=4), *egc* (n=7), *sec* (n=3), and *sel* (n=3) (Table 3).

Table 1. *MIC by broth microdilution (MBD) and performance of Microscan, Vitek, and Phoenix*

	Broth Microdilution				Microscan			Vitek		Phoenix		
mg/L	Oxacillin	Cefuroxime	Ceftriaxone	Meropenem	Oxacillin	Cefoxitine	Cefotaxime	Oxacillin	Cefoxitin	Oxacillin	Cefoxitine	Moxalactam
0.25				1	18			1**		1**		
0.5	1			7	38		1**	13		3		
1	0			14	10			26		15	1**	
2	4	2		20	6			14		41	11	
4	11	7		17		2**		18*	3 negative	12*	48	
8	37	12	3	6		70*			69 positive		12*	
16	16	16	17	4			71					5
32		10	37									67**
64		7	11									
128		8	1*									
> 128		7										

** ≥ ; ** ≤* *Break points: Oxacillin > 2 mg/L Cefoxitin > 4 mg/L*

Table 2. Performance of MRSA screening media: MRSA Select (BioRad), Brilliance MRSA 2 (Oxoid), ChromID MRSA (bioMérieux), BBL CHROMagar MRSA II (Becton Dickinson)

			MRSA select		MRSA Brilliance 2		chromID MRSA		CHROMAgar II	
Clonal Complex	*Spa* types	*agr*	24 h Sens/Res	48 h Sens/Res	24 h Sens/Res	48 h Sens/Res	24 h Sens/Res	48 h Sens/Res	24 h Sens/Res	48 h Sens/Res
CC130 n=60	t843, t528, t1048, t1532, t1535, t3218, t3256, t3570, t5970	*agr* 3	28/35	5/55	0/60	0/60	0/60	0/60	9/51	0/60
CC1943 n = 12	t978, t2345, t3391, t8835	*agr* 4	7/5	2/10	0/12	0/12	0/12	0/12	3/9	0/12
Correctly identified			55.6%	90.3%	100%	100%	100%	100%	83.3%	100%

Table 3. agr type, resistance profile and toxin gene profile

	Number of strains	Resistance gene profiles	Toxin gene profiles
agr 3 n=20	18	*tet* Efflux	all negative
	2	*fos*B, *tet* Efflux	
agr 4 n=7	3	*tet* Efflux	*tst, sec, seg, sei, sel, sem, sen, seo, seu*
	1		*tst, seg, sei, sem, sen, seo, seu*
	3		*seg, sei, sem, sen, seo, seu*

Table 4. PBP2a immunoassay

	Clearview Exact PBP2a (Alere)		PBP2a agglutination (Oxoid)	
	Without FOX induction	With FOX induction	Without FOX induction	With FOX induction
agr 3 n=60	10	60	1	1
agr 4 n=12	0	12	0	0
Correctly identified	14%	100%	1.4%	1.4%

CONCLUSION

The data presented demonstrates that cefoxitin disk diffusion reliably detect isolates with the novel *mecA*$_{LGA251}$ gene. The ability of the different commercial systems to detect these isolates is, however, highly variable and *mecA*$_{LGA251}$ isolates may be missed depending on the algorithms used.

None of the currently available commercial genotypic methods is able to detetect *mecA*$_{LGA251}$ isolates. The only ways to confirm the presence of a supplementary PBP in isolates with MRSA phenotype but no *mecA* gene detected, are the use of specific *mecA*$_{LGA251}$ PCR or Clearview exact PBP2a assay after cefoxitin induction.

KEY POINTS

➔ chromID® MRSA is able to detect *mecA*$_{LGA251}$, unlike molecular biology methods.

Effectiveness of chromID™ MRSA medium for use in clinical nasal specimens

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INTRODUCTION

Staphylococcus aureus (SA) is a major nosocomial pathogen that causes a range of disease including endocarditis, osteomyelitis, toxic shock syndrome, food poisoning, carbuncles and boils. In the early 1950s, acquisition and spread of beta-lactamase-producing plasmids thwarted the effectiveness of penicillin for treating SA infections. In 1959, methicillin, a synthetic penicillin, was introduced. However, by 1960, methicillin resistant *S. aureus* (MRSA) strains were identified, the direct results of SA acquiring the *mec*A gene.

Nosocomial infections caused by MRSA now represent the majority of SA strains and are responsible for increased hospital stay, costs and mortality. There are also rising cost concerns for MRSA infections. In attempts to limit the spread of these infections, control strategies and policies have begun to be put into place. Controlling MRSA is a primary focus of most hospital infection control programs. Rapid identification (ID) of MRSA will affect patient treatment, prevent transmission and control outbreaks and will represent a definite advantage for infection control programs[1,2,3,4,5,6,7].

bioMérieux, Inc. has developed a chromogenic medium for the screening of MRSA in nasal specimens from chronic carriers or patients who are at risk for MRSA. The purpose of this study was to demonstrate the safety and effectiveness of chromID MRSA as used in a clinical setting.

METHODS

Redundant nasal swab specimens were inoculated to Trypticase Soy Agar with 5% sheep blood (TSAB) and then chromID MRSA. Both agar plates were incubated aerobically at 35°C and the chromID MRSA was incubated in the dark. Plates were examined at 24 and 48 hours for the characteristic presence of cream to white colored beta-hemolytic colonies on TSAB, and green colored colonies on chromID MRSA agar plates.

Figure 1 shows the testing algorithm utilized for this study. Identification of MRSA was confirmed by Gram stain, catalase and a latex agglutination test. MRSA was confirmed by the Cefoxitin Screen test and *mec*A PCR.

Quality control isolates (20 replicates of *Staphylococcus aureus*, ATCC 43300, and *Staphylococcus aureus*, ATCC 29213), reproducibility isolates (10 well characterized strains) and 196 clinical nasal swabs were tested. Reproducibility tests were performed in triplicate daily for 3 days. The sensitivity/specificity and positive/negative predictive values (PPV/NPV) were calculated for detection of MRSA on chromID MRSA and TSBA at 24 & 48 hrs.

RESULTS

The sensitivity/specificity for detection of MRSA compared to conventional ID and susceptibility testing for chromID MRSA was 97.4% and 100%, while the PPV/NPV was 100% and 96.5%, at both 24 & 48 hours. The sensitivity/specificity for detection of MRSA compared to conventional ID and susceptibility testing for TSBA was 91.2% and 100%, while the PPV/NPV was 100% and 89.1% at 24 hours. One additional sample was MRSA positive on TSAB at 48 hours increasing specificity to 90.1% and PPV to 91.9%.

Overall percent agreement for chromID MRSA quality control and reproducibility was 100% and 96.7% at 24 hrs.

Conventional Test Methods (based on results from either test medium):
ID = SA Cefoxitin Screen = R *mec*A PCR = Positive

Table 1. Overall Percent Agreement of chromID MRSA vs Conventional Test Methods at 24 & 48 Hours

	Conventional Pos	Neg	TOTAL
chromID MRSA Pos	111	0	111
chromID MRSA Neg	3	82	85
TOTAL	114	82	196

chromID MRSA performance same at 24 & 48 hours
Sensitivity= 97.4%, Specificity=100%, Positive Predictive Value=100%, Negative Predictive Value=96.5%

Table 2. Overall Percent Agreement of TSAB vs Conventional Test Methods at 24 Hours

	Conventional Pos	Neg	TOTAL
TSAB Pos	104	0	104
TSAB Neg	10	82	92
TOTAL	114	82	196

Sensitivity= 91.2%, Specificity=100%, Positive Predictive Value=100%, Negative Predictive Value=89.1%

Table 3. Overall Percent Agreement of TSAB vs Conventional Test Methods at 48 Hours

	Conventional Pos	Neg	TOTAL
TSAB Pos	105	0	105
TSAB Neg	9	82	91
TOTAL	114	82	196

Sensitivity= 92.1%, Specificity=100%, Positive Predictive Value=100%, Negative Predictive Value=90.1%

Table 4. Overall Percent Agreement of chromID MRSA vs TSAB at 24 & 48 Hours

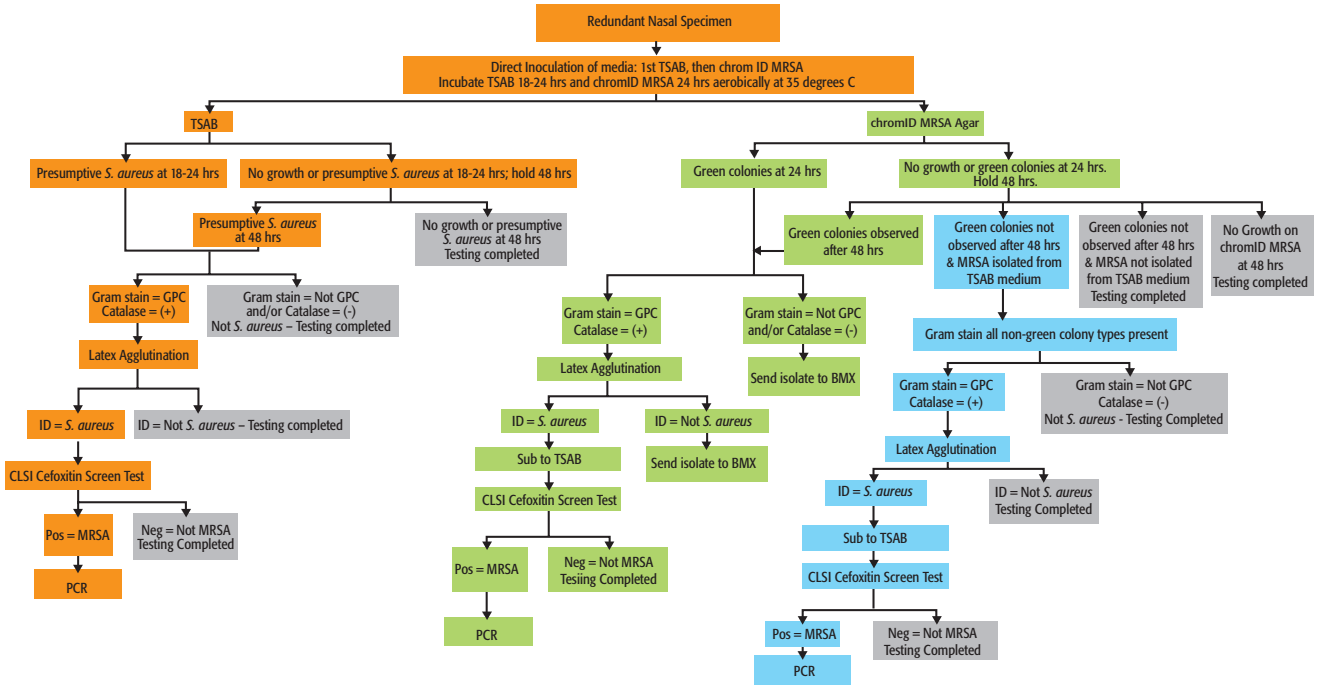
	TSAB Pos	TSAB Neg	TOTAL
chromID MRSA Pos	101/102	10/9	111
chromID MRSA Neg	3	82	85
TOTAL	104/105	92/91	196

Note: one additional MRSA positive sample detected by TSAB at 48 hours; chromID MRSA detection same at 24 & 48 hours incubation. Sensitivity= 97.1%, Specificity=89.1%/90.1%, Positive Predictive Value=91.0%/91.9%, Negative Predictive Value=96.5%

CONCLUSION

This evaluation provides convincing evidence that the performance of chromID MRSA is an excellent screening method with nasal swab specimens for detecting methicillin-resistant *Staphylococcus aureus* in the clinical laboratory and can provide accurate results within 24 hours.

Figure 1. Testing Procedures for chromID MRSA and TSBA AGAR from Clinical Nasal Swab Specimens.



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KEY POINTS

- → chromID® MRSA enables accurate results within 24 hours.
- → chromID® MRSA is highly sensitive and specific for the detection of MRSA compared to conventional ID and susceptibility testing.

chromID® CARBA

chromID® CARBA agar* is a selective chromogenic medium for the screening of Carbapenemase-Producing *Enterobacteriaceae* (CPE), particularly KPC and NDM-1, in patients who are chronic carriers or in patients at risk.

CPE are particularly multi-resistant bacteria that are capable of causing nosocomial infections and hospital epidemics. The detection of CPE carriers is particularly important for the prevention and epidemiological monitoring of these infections. In this context, the use of chromID® CARBA agar contributes to the active surveillance of CPE.

chromID® CARBA agar (patent pending) consists of a rich nutritive base combining different peptones. It contains:
- a mixture of antibiotics which enable the selective growth of CPE.
- three chromogenic substrates which enable the identification of the most frequently isolated CPE.

chromID® OXA-48

chromID® OXA-48 agar* is a selective chromogenic medium for the screening of OXA-48 Carbapenemase-Producing *Enterobacteriaceae* (CPE) in patients who are chronic carriers or in patients at risk.

OXA-48 CPE are particularly multi-resistant bacteria that are capable of causing nosocomial infections and hospital epidemics. The detection of OXA-48 CPE carriers is particularly important for the prevention and epidemiological monitoring of these infections. In this context, the use of chromID® OXA-48 agar contributes to the active surveillance of CPE.

chromID® OXA-48 agar (patent pending) consists of a rich nutritive base combining different peptones. It contains:
- a mixture of antibiotics which enable the selective growth of OXA-48 CPE.
- three chromogenic substrates which enable the identification of the most frequently isolated CPE.

* See package insert for more information

CONTENTS

➔ ARTICLES

➔ POSTERS

EUROPEAN JOURNAL OF CLINICAL MICROBIOLOGY AND INFECTIOUS DISEASES
2013;DOI 10.1007/s10096-013-1925-6

Performance of chromID® CARBA Medium for Carbapenemases-Producing Enterobacteriaceae Detection during Rectal Screening

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Rapid identification of patients colonized by carbapenemases-producing Enterobacteriaceae (CPE) is essential for implementation of infection control precautions. To implement preventive measures and control the spread of CPE, chromogenic chromID® CARBA medium was compared with two culture-based screening methods (CDC procedure and MacConkey agar with imipenem (MCI)) for its performance in detecting carbapenemase-producing Enterobacteriaceae (CPE) during a faecal screening surveillance program.

Double rectal swabs were collected from patients hospitalized in the ICU on admission and every 5-7 days during hospitalization. One swab was directly inoculated onto the solid media chromID® CARBA plate and MacConkey agar with imipenem, while the other was tested according to CDC protocol.

Suspected colonies from all procedures were identified to species level and tested for their susceptibility to carbapenems by phenotypic tests. All carbapenem non-susceptible isolates were tested by the Modified Hodge Test (MHT) and synergy tests. Positive results were confirmed by PCR testing for carbapenemase gene detection. The performance of all three procedures was statistically analyzed as compared to MHT and PCR results for the presence of carbapenemase-encoding genes.

Out of 177 rectal samples tested, 86 samples were found to contain one or more CPE verified by molecular detection of carbapenemase-encoding genes among isolated Enterobacteriaceae. Sensitivity of chromID® CARBA and CDC methods was similarly high for CPE in clinical samples (96.5% and 98.8% respectively) compared to MCI (89.5%). chromID® CARBA had higher specificity before and after Gram staining (91.2% and 100% respectively) compared to the other two media (80.2% and 80.2% for CDC; 31.9% and 70.3% for MCI).

chromID® CARBA performed with high accuracy among the phenotypic methods applied, giving early results.

KEY POINTS

- → chromID® CARBA demonstrated high accuracy, when applied for CPE screening in rectal swabs, with high sensitivity and specificity.
- → chromID® CARBA can therefore be recommended for routine application in surveillance and infection control practices.

DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
2013;75:187-91

Prevalence and molecular characterization of Enterobacteriaceae producing NDM-1 carbapenemase at a military hospital in Pakistan and evaluation of two chromogenic media

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(c) University of Queensland Centre for Clinical Research, Queensland, Australia
(d) School of Life Sciences, University of Northumbria, Newcastle Upon Tyne, NE1 8ST, UK

The aim of this study was to evaluate the performance of 2 chromogenic media (chromID CARBA and Brilliance CRE) recommended for isolation of carbapenemase-producing *Enterobacteriaceae* (CPE) in stool samples from patients attending a military hospital in Rawalpindi, Pakistan. Further aims included the identification of factors that might predispose to faecal carriage of CPE and to assess the prevalence and genotypic diversity of CPE in this population.

One hundred and seventy-five stool samples were collected from distinct patients attending the military hospital (143 on surgical wards and 32 outpatients). Of the 175 patients, 32 (18.3%) had faecal carriage of CPE and all produced NDM-1 carbapenemase. All of these 32 patients were detected using chromID CARBA compared with 20 patients (62.5%) detected using Brilliance CRE ($P = 0.0015$). If only colored colonies were considered as presumptive CPE, chromID CARBA also showed very high specificity (98%) with only 5 false-positive isolates of *Enterobacteriaceae* recovered from 175 samples.

In this study, duration of hospitalization and treatment with coamoxyclav were statistically associated with a higher likelihood of carriage of CPE ($P \leq 0.05$). The majority of NDM-1–producing *Enterobacteriaceae* co-produced CTX-M-1 group extended spectrum β-lactamase (ESBL), and one third produced armA-type methylase. NDM-1 carbapenemase was most commonly found amongst commensal types of Escherichia coli, especially phylogenetic group B1.

KEY POINTS

→ The selectivity of chromID® CARBA is crucial for CPE screening especially when ESBL and /or AmpC ß-lactamase *Enterobacteriaceae* are endemic.

JOURNAL OF CLINICAL MICROBIOLOGY
2012;50:3102-4

A comparison of four chromogenic culture media for carbapenemase-producing Enterobacteriaceae

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(b) Microbiology Department, Royal Hallamshire Hospital, Sheffield, United Kingdom
(c) School of Life Sciences, University of Northumbria, Newcastle upon Tyne, United Kingdom

The aim of this study was to evaluate the suitability of chromogenic media recommended for isolation of carbapenemase-producing *Enterobacteriaceae* (CPE).

Four chromogenic media and two selective broths were challenged with a collection of *Enterobacteriaceae* with well-defined β-lactamases and 100 stool samples.

With low inoculum of 130 isolates of CPE, the sensitivities of the four chromogenic media were: Brilliance CRE, 78%; chromID Carba, 91%; chromID ESBL, 96%; and Colorex KPC, 56%. The corresponding sensitivities of Trypticase soy broth plus ertapenem or meropenem were 78% and 47%, respectively.

ChromID Carba showed optimal performance for both sensitivity (91%) and specificity (89%) for the detection of CPE with the collection of isolates used in this study.

KEY POINTS

➔ chromID® CARBA is the most specific chromogenic medium for CPE screening with a high level of sensitivity.

Evaluation of chromID CARBA agar medium (bioMérieux) performance for the detection of Carbapenemase-producing Enterobacteriaceae

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INTRODUCTION AND PURPOSE

Carbapenems are used as a last-resort antibiotic class for the treatment of infections due to multidrug-resistant *Enterobacteriaceae*. However, during the last decade, carbapenem resistance has been increasingly reported and carbapenemase-producing *Enterobacteriaceae* (CPE) are emerging as a growing challenge in health care facilities (1).

The clinically significant carbapenemases in *Enterobacteriaceae* belong mostly to: Ambler class A (KPC), the zinc-dependent class B (NDM, VIM, and IMP) and the class D (OXA-48-like) of β-lactamases (2).

Carbapenemase-producing pathogens have been associated with high rates of morbidity and mortality particularly among critically ill patients with prolonged hospitalization. It is also of note that the carbapenemase genes harbored by CPE are mostly transposon- and/or integron-encoded determinants that can easily disseminate to other enterobacterial strains and species.

These facts suggest the need to implement adequate preventive measures, including active surveillance, in order to detect infected patients and carriers with multidrug-resistant isolates and contain the spread of these pathogens. (3)

The direct detection of CPE carriers, by selective chromogenic medium, is a useful tool for rapid and inexpensive screening of patients for CPE and is also available for daily use in many laboratories.

Culture techniques for screening CPE have been tested, including methods that use in-house-prepared selective media, such as TSBs containing a 10 µg carbapenem disk or selective chromogenic agar media, like chromID CARBA agar.

ChromID CARBA agar is designed for CPE detection and is supplemented with a mixture of antibiotics that inhibit the growth of Gram-positive and non-CPE (4) and with three chromogenic substrates that may contribute to the recognition of enterobacterial species: *Escherichia coli* produce pink to burgundy colonies or translucid colonies with a pink to burgundy center, while *Klebsiella, Enterobacter, Serratia, Citrobacter* (KESC group) species produce bluish-green to bluish-grey colonies.

The aim of this study was to evaluate the performance of chromID CARBA agar medium, provided by bioMérieux (Marcy l'Etoile, France) for: i) detection and differentiation of a well previously characterized collection of CPE with various enzymatic resistance mechanisms and ii) screening for CPE carriers.

METHODS

Overall 60 CPE (47 KPC-, 11VIM-type, 1 OXA-48 and 1 NDM-1) isolates and, as negative control, 40 non-CPE susceptible to carbapenems or resistant with mechanism other than carbapenemase production (24 CTX-M-type, 8 TEM-type, 6 CMY-16, 2 porin loss plus ESBL or AmpC hyper-producing isolates) were plated onto chromID CARBA medium. Two different inoculum sizes (10^5 CFU/µl and 10^2 CFU/µl) of each isolate were plated onto the medium. All plates were incubated at 37°C and inspected for growth and colony colour after 18, 24, and 48h.

To validate the method of inoculum preparation, 10 strains were selected at random, 1 µl of the diluted suspension was inoculated onto each of three Columbia blood agar plates, and after incubation for 24 h in air at 37°C, the average number of colonies for each isolate was recorded.

The performance of CARBA agar medium was compared with the screening method recommended by CDC (5). Each of the suspensions described above was further diluted 1/20 in saline, and 100 µl of each was used to inoculate 5 ml of TSB containing a 10 µg ertapenem disc and 5 ml of TSB containing a 10 µg meropenem disc. The broths were incubated for 18 h at 37°C, and 100 µl of broth was then cultured onto MacConkey agar, which was incubated for 18 h at 37°C.

The medium was assessed for their ability to inhibit the growth of other commensal microorganisms non-CPE in mixed cultures.

Mixed cultures were prepared in different ratios of CPE/non-CPE (1:100; 1:1,000; 1:10,000) in saline, 1 µl of the suspension was inoculated onto chromID CARBA agar. All plates were incubated in air at 37°C and the average number of colonies was recorded after 24h.

Four hundred rectal swabs from laboratory routine were directly plated onto chromID CARBA agar medium in two different Italian laboratories. The results were compared to standard routine laboratory method for CPE screening. All different colonies recovered from chromID CARBA agar were subjected to identification with MALDI-TOF (Vitek-MS, bioMérieux).

Figure 1.

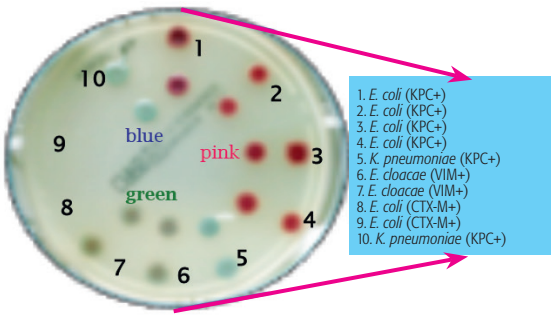
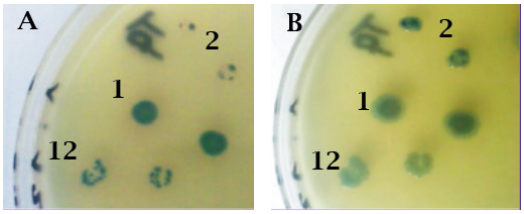


Figure 2.

A. After 24h of incubation; B. After 48h of incubation.

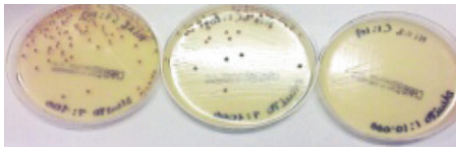


12) E. cloacae (porin loss)
1) E. cloacae (VIM+)
2) K. pneumoniae (porin loss)

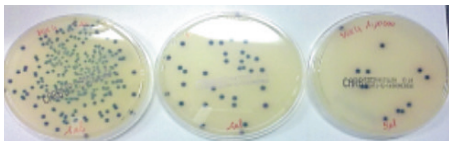
Evaluation of chromID CARBA agar medium (bioMérieux) performance for the detection of Carbapenemase-producing Enterobacteriaceae

Figure 3. *Mixed cultures in different ratios of CPE/non-CPE (1:100; 1:1,000; 1:10,000)*

E. coli (KPC+)/ K. pneumoniae (CTX-M+)



K. pneumoniae (KPC+)/ E. coli (CTX-M+)



RESULTS

All CPE strains grew on chromID CARBA agar medium after 18h of incubation, independently of inoculum size, and developed characteristic coloration of species (Fig. 1). No growth was detected for control non-CPE isolates except for the two carbapenem resistant porin loss isolates that grew after 24h of incubation (Fig. 2). Colony counts, performed on 10 isolates, to validate the method of inoculum preparation revealed an average count approximately of 10^5 CFU/spot and 10^2 CFU/spot for high and low inoculum, respectively.

Out of the 400 rectal swabs analysed, 32 were positive with both routine and chromID CARBA agar methods and 2 were positive only for the latter. These two isolates were from patients with a low level of colonization (<4 colonies) and were confirmed as KPC-producers with molecular methods. In 11 cases a growth of non-enterobacteriaceae organisms was detected (2 *Aeromonas* spp., 10 *Pseudomonas* spp., 1 *Stenotrophomonas maltophilia* and 1 *Enterococcus faecium*).

chromID CARBA agar medium showed high sensitivity allowing to isolate CPE strains from mixed cultures also in minority concentration (Fig. 3).

CONCLUSIONS

ChromID CARBA agar medium demonstrated good performance with the collection of CPE used, and also for CPE difficult to detect due to the low carbapenems MIC, such as OXA-48 and VIM-type producing strains. Extended incubation for 48h had impact on the specificity of chromID CARBA agar medium as two carbapenem resistant porin loss isolates grew after incubation for up to 24h (Fig. 2).

ChromID CARBA agar medium showed a high correlation with the routine procedures and in 2 cases a higher sensitivity in the detection of CPE. Further, this method allows the isolation of CPE strains from mixed cultures including at lower concentrations.

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KEY POINTS

- → chromID® CARBA allows isolation of CPE strains from mixed cultures.
- → chromID® CARBA shows better recovery than routine methods in cases of low CPE bacterial load.

High prevalence of NDM-1 producing bacteria in patients from Bangladesh detected by chromID® CARBA

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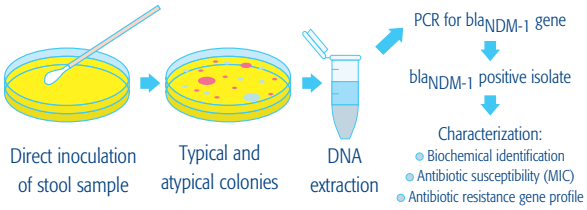
INTRODUCTION AND PURPOSE

The increasing incidence of infections caused by NDM-1-producing *Enterobacteriaceae* is a major worry to the international medical community. We reported previously that approximately 3.5% of Gram negative clinical isolates in Bangladesh were NDM-1-producing (Islam et al., 2012). The main objective of the present study was to determine the prevalence of fecal carriage of NDM-1-producing organisms among hospitalized patients with diarrhoea and outpatients attending Dhaka hospital of icddr,b in Bangladesh by direct inoculation of stool samples on chromID™ CARBA agar, a new chromogenic culture medium for isolation of carbapenemase-producing organisms.

METHODS

Stool samples from 100 patients were directly inoculated on chromID™ CARBA agar. Of the 100 stool samples, 50 were collected from outpatient services of icddr,b (OP sample) and 50 from patients with diarrhoea under 2% surveillance (every 50th patient) of icddr,b Dhaka hospital (HS sample). Stool samples were collected consecutively from September to October 2012. Methodology used in the study has been described in Fig.1.

Figure 1 *Flow diagram of methodology used in the study*



RESULTS

A total of 34 samples (13 OP and 21 HS) showed growth on chromID™ CARBA agar plates of which 20 (10 OP and 10 HS) had typical colonies (pink to burgundy/ bluish-green to bluish-grey) and the remaining 14 samples had colonies with atypical colors (white/ light brown) (Fig. 2, Table 1). Seven of 20 samples with typical colonies were positive for blaNDM-1. Two OP samples that showed atypical colonies were also positive for blaNDM-1. Overall, 9% (n=9) of the samples were positive for NDM-1-producing organisms. The NDM-1 prevalence in patients from outpatient services was higher (14%, n=7) as compared to patients from the hospital surveillance (4%, n=2). A total of 19 strains was isolated of which 9 were identified as *E. coli*, 6 as *Klebsiella pneumoniae* and one as *Pantoea* spp. Three strains could not be identified by using API® 20E system (Table 1). All strains were resistant to multiple antibiotics though some remained susceptible to minocycline (13/19), amikacin (9/19), gentamicin (4/19) and aztreonam (2/19) (Table 2). All were susceptible to tigecycline and colistin. All 19 strains were positive for bla_{TEM}, 6 for bla_{SHV}, 14 for $bla_{CTXM\text{-}group\ 1}$, 16 for $bla_{CTXM\text{-}15}$, 18 for $bla_{OXA\text{-}1}$. Only 2 strains were positive for *qnrB*, 2 strains each were positive for *rmtB* and *rmtC* and one strain for *arm*A encoding 16s rRNA methylase (Table 3).

Figure 2. *Colony morphology of bacteria on chromID™ CARBA agar plates. A, mixed culture predominating typical K. pneumoniae colonies; B, typical E. coli colonies; C, mixed culture*

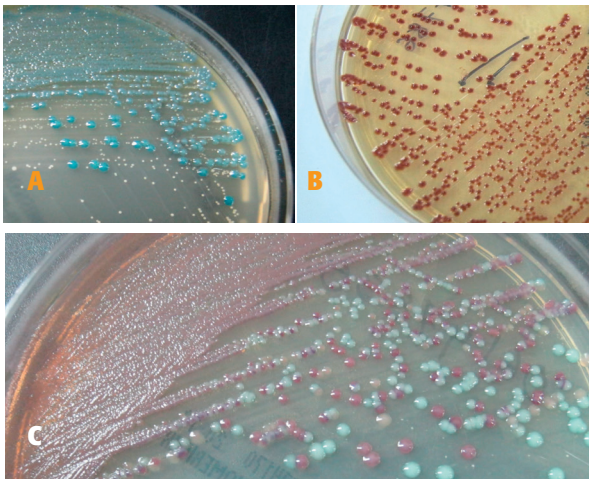


Table 1 *Colony characteristics and biochemical identification of 19 NDM-1 positive isolates recovered from stool samples of 7 outpatients and 2 hospitalized patients at icddr,b hospital*

Sample source	Strain ID	blaNDM-1	Colony Char. on chomID™ CARBA	API® 20E
Outpatient	7762C1	+	Bluish-green	*K. pneumoniae*
	7762C2	+	Bluish-green	*K. pneumoniae*
	7762C3	+	Bluish-green	*K. pneumoniae*
	7773C2	+	Burgundy	*E. coli*
	7773C3	+	Burgundy	*E. coli*
	7819C1	+	Bluish-green	*K. pneumoniae*
	7819C2	+	Bluish-green	*K. pneumoniae*
	7826C1	+	Light brown	*E. coli*
	7826C3	+	Light brown	*E. coli*
	7840C1	+	Burgundy	*E. coli*
	7840C2	+	Burgundy	*E. coli*
	7840C3	+	Burgundy	*E. coli*
	7845C2	+	Light brown	*E. coli*
	7845C3	+	Light brown	*E. coli*
	7849C2	+	Bluish-green	unidentified
	7849C3	+	Bluish-green	unidentified
Hospital surveillance	6650C1	+	Bluish-green	*Pantoea* spp
	6650C3	+	White	unidentified
	6600C1	+	Bluish-green	*K. pneumoniae*

High prevalence of NDM-1 producing bacteria in patients from Bangladesh detected by chromID® CARBA

Table 2 Antibiotic susceptibility of the 19 NDM-1 positive bacterial isolates isolated from 9 stool samples

Antibiotic	MIC_{50} (mg/L)	MIC_{90} (mg/L)	MIC range	% resistant
ceftriaxone	>256	>256	>256->256	100
aztreonam	>256	32	0.13->256	90
pipera/tazo	>256	>256	>256->256	100
imipenem	>32	16	3->32	95
ceftazidime	>256	>256	96->256	100
cefotaxime	>256	>256	>256->256	100
minocycline	4	1.5	1.5-48	32
colistin	1.5	1.0	1-6	0
tigecycline	0.38	0.25	0.19-2.0	0
gentamicin	>256	2	1->256	79
meropenem	>32	16	3->32	90
tobramycin	24	3	3->256	63
amikacin	>256	3	3->256	53
cefpirome	>256	>256	>256->256	100

We report a high prevalence (9%) of fecal carriage of NDM-1-producing organisms in patients from Bangladesh. The figure is higher as compared to prior reports from our group where we reported a prevalence of NDM-1 among Gram-negative bacteria from clinical samples of 3.5%. Compared to methods that we used in our previous studies (screening of carbapenem resistant organisms using MHA plates containing imipenem, followed by PCR testing of selected colonies for NDM-1 gene) the direct plating of sample on chromID™ CARBA agar is a more convenient method by which target organisms can be easily identified by their distinct colony morphologies.

REFERENCES

Islam MA, Talukdar PK, Hoque A, et al. Emergence of multidrug resistant NDM-1-producing Gram-negative bacteria in Bangladesh. Eur J Clin Microbiol Infect Dis. 2012; 10:2593-2600.

Table 3 Antibiotic resistance gene profiles of 19 NDM-1 positive bacterial isolates isolated from stool samples

Strain ID	*bla*TEM	*bla*SHV	*bla*CTXM conserved	*bla*CTXM gr-I	*bla*CTXM-15	*bla*OXA-1	*bla*OXA-47	*qnr*A/B/S	*rmt*B/C/*arm*A
7762C1	+	+	+	+	+	+	+	*qnr*B	*arm*A
7762C2	+	+	+	+	+	+	+	*qnr*B	-
7762C3	+	+	-	-	-	-	-	-	-
7773C2	+	-	+	+	+	+	+	-	*rmt*B
7773C3	+	-	+	+	+	+	+	-	-
7819C1	+	+	+	+	+	+	-	-	-
7819C2	+	+	+	+	+	+	+	-	-
7826C1	+	-	+	+	+	+	+	-	-
7826C3	+	-	+	+	+	+	+	-	-
7840C1	+	-	+	+	+	+	+	-	-
7840C2	+	-	+	+	+	+	+	-	-
7840C3	+	-	+	+	+	+	+	-	-
7845C2	+	-	+	+	+	+	+	-	-
7845C3	+	-	+	+	+	+	+	-	-
7849C2	+	-	+	-	+	+	+	-	-
7849C3	+	-	+	-	+	+	+	-	-
6650C1	+	-	-	-	-	+	+	-	*rmt*C
6650C3	+	-	-	-	-	+	+	-	*rmt*C
6600C1	+	+	+	+	+	+	+	-	-

KEY POINTS

→ chromID® CARBA shows the capability to detect NDM-1 producing *Enterobacteriaceae* from stool samples.

First evaluation of chromID® OXA-48 agar - a new chromogenic medium for detection of Enterobacteriaceae-producing OXA-48 carbapenemase

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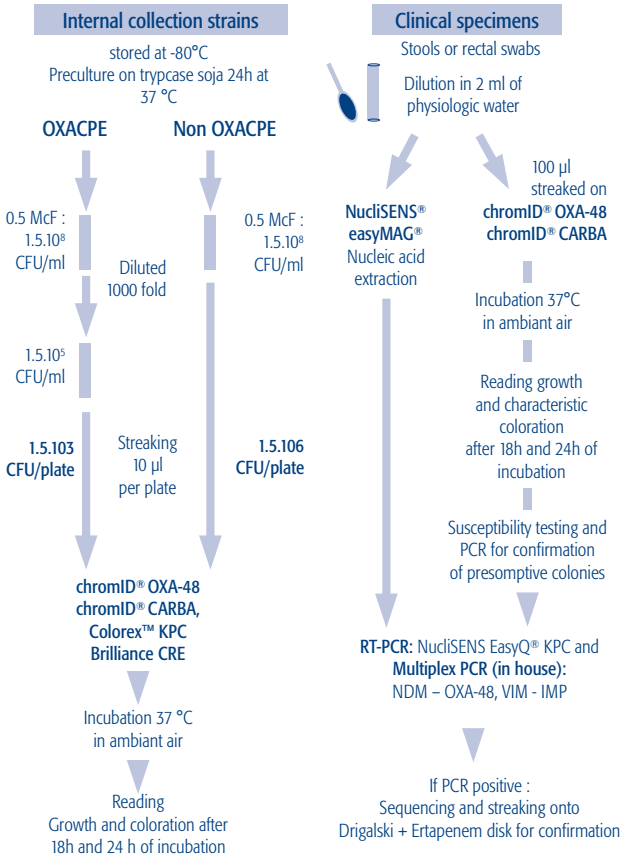
INTRODUCTION

The spreading of strains producing OXA-48 carbapenemase is an emerging public health problem, particularly in Europe and throughout the Mediterranean. These strains are responsible for outbreaks and are often difficult to detect because of their low level of resistance expression. The purpose of this study is to evaluate the performance of chromID® OXA-48, a new chromogenic medium developed for the specific detection of *Enterobacteriaceae* (EB) producing OXA-48 carbapenemase (OXACPE). To assess the sensitivity and specificity, this medium was compared with internal collection strains to other available chromogenic media dedicated to the detection of carbapenemase producing strains (CPE): chromID®CARBA (bioMérieux), Brilliance CRE (Oxoid) and Colorex™KPC (Diagnostics BioMed). Specificity was also assessed with clinical specimens plated on the chromID®OXA-48 and chromID®CARBA. Status of clinical samples was defined by direct PCR testing.

METHOD

Figure 1. Protocol



Principle

the chromID® OXA-48 medium allows selective detection of the OXACPE by a coloration of the colonies thanks to the use of chromogenic substrates.

Reading and interpretation

- positive OXACPE: growth with characteristic coloration of the colonies (see figure 2)
- negative: no growth, growth of colourless colonies or colonies without characteristic coloration.

chromID® CPS was used as growth control for internal collection strains. The other media were used according to the supplier instructions.

- All the collection strains were characterized by molecular or phenotypic methods (see table 1).

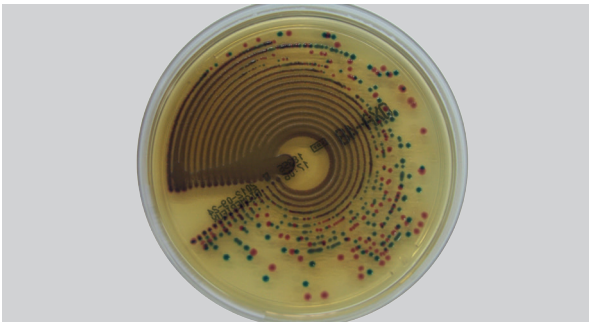
Clinical sample

- Origin: Hospital centres from Aix-en-Provence, Alès, Aubagne, Montpellier, Nîmes, Perpignan and Toulouse (France)
- 1135 Specimens: 774 stools and 361 rectal swabs were collected from February 1 to April 30, 2012.

Table 1. Distribution of the internal collection strains

33	EB producing OXA-48 type carbapenemase *6 Escherichia coli, 4 Enterobacter cloacae, 23 Klebsiella pneumoniae*
4	EB producing OXA-181 type carbapenemase (OXA-48 variant) *3 Klebsiella pneumoniae, 1 Providencia rettgeri*
35	EB producing other carbapenemase *1 IMI, 6 IMP, 11 KPC, 10 NDM, 3 SME, 4 VIM*
36	EB producing ESBLor gram negative bacteria over producing cephalosporinase (HLCase) *14 HLCase, 22 ESBL*
14	EB carbapenem resistant by impermeability
10	Non fermentative Gram negative OXA-23
56	Wild type strains: *12 Enterobacteriaceae* *8 Candida spp* *4 Staphylococcus aureus, 3 Enterococcus* non fermentative gram - bacteria: *11 Acinetobacter baumanii, 11 Pseudomonas aeruginosa* *7 Stenotrophomonas maltophillia*
14	Vancomycin Resistant Enterococci, Van A or Van B

Figure 2. Mixed culture of OXACPE: K. pneumoniae (green colonies) E. coli (pink colonies)



First evaluation of chromID® OXA-48 agar - a new chromogenic medium for detection of Enterobacteriaceae-producing OXA-48 carbapenemase

Table 2: Number of internal collection strains detected as positive on the different media

		chromID® OXA-48		chromID® CARBA		Colorex KPC		Brilliance CRE	
Nb of strains		18h	24h	18h	24h	18h	24h	18h	24h
37	OXA-48 / OXA-181	37	37	15	15	18	20	20	20
35	Other carbapenemase	1	1	34	34	32	34	24	24
36	HL Case, ESBL	-	-	-	2	1	1	9	9
14	resistant by impermeability	-	-	6	9	12	12	10	10
10	Other oxacillinase (OXA-23)	-	-	-	-	-	-	-	-
56	wild type	-	-	-	3	1	1	1	2
14	VRE	1	1	5	7	-	-	-	-
202	total								

Table 3: Performance of the different chromogenic media solution for CPE detection

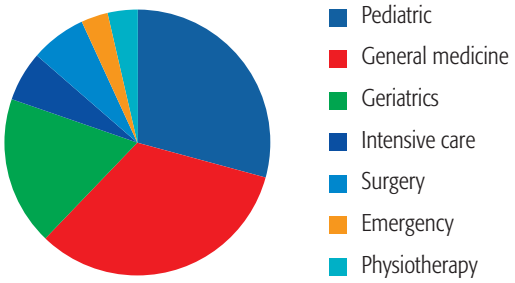
	Combination chromID® OXA-48 + chromID® CARBA		Colorex KPC		Brilliance CRE	
	18h	24h	18h	24h	18h	24h
Fertility in %	100	100	74	76	75	75
Sensitivity in %	99	99	69	75	61	61
Specificity in %	92 (95)	84 (89)	89 (89)	89 (89)	85 (85)	84 (84)

(x): performance after gram staining

Table 4: Clinical samples: growth and PCR results

	chromID® OXA-48		chromID® CARBA		PCR	Drigalski + ertapenem disk
1135 clinical stools or rectal swabs	18h	24h	18h	24h		
OXA-48	1	1	1	1	3	1
Other carbapenemase	-	-	-	-	-	-
P. Aeruginosa Wild Type	-	-	33	33		
P. Aeruginosa OprD loss	-	-	2	2		
P. Aeruginosa OprD loss + Efflux	12	12	14	14		
HL case, ESBL and porin loss	2	2	2	2		
Colourless colonies	14	14	15	15		

Figure 3 : Distribution of the origin of the clinical specimens



DISCUSSION

Detection of OXACPE: All tested strains were detected after 18 hours of incubation on the chromID® OXA-48 medium. The sensitivity of this medium is excellent despite the low inoculum used (1.5 10^3 CFU/plate). The specificity of detection of chromID® OXA-48 is also very high as 98% of the non-OXACPE tested were negative: the 2 false-positive strains growing on the medium were: (i) *S. marcescens* producing an IMP carbapenemase, and (ii) one VRE (*E. faecium*) that could easily be differentiated from EB by morphology, colonies coloration and/or Gram staining.

Detection of all CPE: The combination of chromID® OXA-48 and chromID® CARBA allows the detection of 99% of CPE with 95% of specificity after 18 hours. The false positive results are due to the growth of VRE on chromID® CARBA (negative after Gram staining) and of some strains resistant to carbapenem by impermeability. In contrast, only 61% and 69% CPE were detected after 18 hours of incubation by Brilliance CRE and Colorex KPC respectively. Most of the false positives on Brilliance CRE and Colorex KPC are due to a lack of selectivity of the 2 media regarding EB strains with porin loss or producing HL Case and ESBL (13 and 19 respectively), false positives that cannot be distinguished from CPE on the basis of morphological characteristics.

Clinical samples: Those results were confirmed by the clinical study. Despite the low prevalence of digestive carriage of OXACPE in the south of France (0.3% in this study), the two media showed excellent specificities, 98.7% for chromID® OXA-48 and 95.5% for chromID® CARBA medium.

CONCLUSION

These studies highlight the high sensitivity and specificity of the chromID® OXA-48 medium for the detection of strains producing OXA-48 carbapenemase. The combination of the ready-to-use media chromID® CARBA and chromID® OXA-48 is the relevant solution which allows the optimal detection of all Enterobacteriaceae-producing carbapenemases, including OXA-48 carbapenemases, after only 18 hours of incubation. This combination of chromID® CARBA and chromID® OXA-48 should facilitate infection control and the prevention of epidemics, even in emerging countries.

KEY POINTS

- → chromID® OXA-48 allows screening of OXA-48 CPE.
- → The association of chromID® CARBA and chromID® OXA-48 allows screening of all relevant carbapenemases (CPE) regardless of the enzyme.

Evaluation of a new chromogenic medium, chromID™ CARBA, for the detection of carbapenemase-producing *Enterobacteriaceae*

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INTRODUCTION AND PURPOSE

Carbapenemase Producing Enterobacteriaceae (CPE) are multi-resistant emerging bacteria which can be responsible for hospital acquired infections and outbreaks. Detection of CPE carriers is of particular importance for prevention and epidemiological monitoring of these infections. In this context, chromogenic media for CPE should make screening easier due to their selectivity and the use of different colours to discriminate targeted species. The aim of this study was to evaluate the performance of two chromogenic media for CPE detection, namely chromID™ CARBA (a prototype medium from bioMérieux based on the same principles than the ones – ID Carba – previously tested by Perry et al., 2011) and CHROMagar™ KPC (CHROMagar). A commonly used selective home-brewed medium, MacConkey supplemented with 1 mg/L imipenem (McC+I), was also included in the study.

METHODS

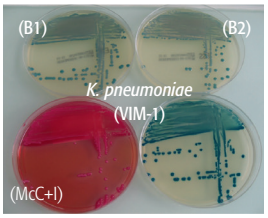
A total of 194 isolates was tested, including 127 CPE with different types of carbapenemases (53 KPC, 44 NDM, 13 VIM, 11 IMP, 6 OXA-48) and 67 isolates not producing a carbapenemase. All microorganisms were inoculated directly onto each medium with 10 µL of a 0.5 McFarland calibrated suspension before 24 h incubation at 34-38°C. Two batches of chromID™ CARBA were used: one freshly prepared (B1) and one close to the expiry date (B2).

RESULTS

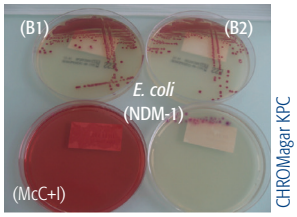
Sensitivity and specificity results for CPE detection are shown in the table below. Typical colonies of *Klebsiella pneumoniae* (coloured in green) and *Escherichia coli* (coloured in pink) are presented for each medium tested. For chromID™ CARBA, the sensitivity for CPE detection varied from 89.8% (B1) to 96.1% (B2).

By comparison, the sensitivity was 89.0% for CHROMagar™ KPC and only 68.5% for McC+I. Variation in sensitivity was dependent on the types of carbapenemases. For KPC, NDM and VIM the sensitivity of detection by the two batches of chromID™ CARBA was similar and usually higher when compared to CHROMagar™ KPC and McC+I. However, detection of IMP and OXA-48 could be more difficult with a fresh medium than with a medium close to the expiry date. Nonetheless, the specificity of chromID™ CARBA (B2) remained stable over time.

chromID™ CARBA



chromID™ CARBA



CONCLUSION

This study highlights the superior sensitivity of both chromogenic media over the imipenem supplemented MacConkey. In comparison to CHROMagar™ KPC, chromID™ CARBA presents three advantages: (i) trend to higher sensitivity, (ii) ready to use plates and (iii) extended shelf life. As such, it has the potential of being a very useful tool for the screening of patients who carry the widespread KPC and NDM-producing *Enterobacteriaceae*. These results should be confirmed with clinical samples such as rectal swabs.

Type	Total	Microorganism (nb of strain)	chromID Carba (B1)	chromID Carba (B2)	CHROM agar KPC	Mac Conkey (McC+1)
Class A KPC	53	*E. coli* (6) *Klebsiella* (43) *Enterobacter* (4)	96.2%	100%	96.2%	56.6%
Class B NDM	44	*E. coli* (31) *Klebsiella* (5) *Enterobacter* (7) *Citrobacter**(1)	97.7%	97.7%	90.9%	77.8%
Class B VIM	13	*E. coli* (5) *Klebsiella* (2) *Enterobacter* (5) *Serratia* (1)	84.6%	92.3%	61.5%	69.2%
Class B IMP	11	*E. coli* (2) *Klebsiella* (1) *Enterobacter*** (7) *Serratia* (1)	45.5%	72.7%	81.8%	81.8%
Class D OXA-48	6	*E. coli* (1) *Klebsiella* (5)	66.7%	100%	83.3%	83.3%
Sensitivity (True positive/ (True positive + false negative) x 100			89.8%	96.1%	89%	68.5%
Specificity (True negative/ (True negative + false positive+) x 100			97%	94%	94%	96%

KEY POINTS

→ chromID® CARBA performance was evaluated at 2 different stages of storage.

→ chromID® CARBA shows better sensitivity to all kinds of CPE compared to other techniques.

chromID® ESBL

chromID® ESBL agar* is a selective chromogenic medium for the screening of Extended Spectrum ß-Lactamase producing *Enterobacteriaceae* in chronic carrier patients or patients at risk.

ESBL-producing *Enterobacteriaceae* are multi-resistant bacteria which can be responsible for nosocomial infections. The detection of ESBL-producing *Enterobacteriaceae* carriers is particularly important for the prevention and epidemiological monitoring of these infections. In this context, the use of chromID™ ESBL agar contributes to the active surveillance of ESBL-producing *Enterobacteriaceae*.

chromID® ESBL agar (patent pending) consists of a rich nutritive base including a variety of peptones. It contains:

- a mixture of antibiotics, including cefpodoxime, enabling the selective growth of ESBL-producing enterobacteria.
- two chromogenic substrates and one natural substrate enabling the direct identification of the most frequently encountered ESBL-producing *Enterobacteriaceae*.

* See package insert for more information

CONTENTS

➔ ARTICLES

➔ POSTERS

ECCMID 2012 / London (United Kingdom)

ECCMID 2009 / Helsinki (Finland)

DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
2010;67:376-379

Assessment of prevalence and changing epidemiology of extended-spectrum beta-lactamase-producing Enterobacteriaceae fecal carriers using a chromogenic medium

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(b) Unidad Asociada al Centro Nacional de Biotecnología, Consejo Superior de Investigaciones Científicas (CSIC) "Resistencia a los antibióticos y virulencia bacteriana", Hospital Universitario Ramón y Cajal, 28034-Madrid, Spain

This study aimed to assess the current level of extended-spectrum β-lactamase (ESBL) fecal carriage in Madrid (Spain) using a commercial chromogenic medium, and to investigate the changing epidemiology of ESBLs in a local geographic area, compared with previous studies.

Five hundred fecal samples from 462 patients (68.4% ambulatory) were screened for ESBL-producers using (i) ceftazidime and cefotaxime (1 mg/L) MacConkey (MAC) agar plates and (ii) a chromogenic media (chromID ESBL; bioMérieux, Marcy-l'Etoile, France).

A prevalence of 8.2% of ESBL fecal carriers was observed (8.9% hospitalized, 7.9% non-hospitalized patients), which was higher than that observed in previous studies (1991, 0.6%; 2003, 7.0%).

Sensitivity, specificity, and positive and negative predicted values were 100%, 94.8%, 63%, and 100% for chromID ESBL and 87.8%, 89.8%, 43.4%, and 98.9% for MAC, respectively. The chromID ESBL medium was reliable to screen ESBL fecal carriers with a general decrease in the laboratory workload.

The study demonstrated a slightly increased rate of ESBLs fecal carriers compared with 2007 in non-hospitalized patients. Time-to-time monitoring of fecal carriers of ESBL-producing isolates and characterization of the corresponding enzymes is shown to be useful to track ESBLs and their changing epidemiology, and to understand current epidemiology of ESBL-producing isolates in clinical samples.

In conclusion, this study showed the selective chromogenic chromID™ ESBL medium to be reliable for the screening of fecal carriers with ESBL-producing Enterobacteriaceae. A general decrease in the workload and a potential reduction of reporting time due to the absence of redundant confirmatory tests can be the advantages of this medium. In addition, it is a useful tool for epidemiologic studies.

KEY POINTS

➔ The use of chromID® ESBL decreases the workload by reducing the number of confirmatory tests.

JOURNAL OF MEDICAL MICROBIOLOGY
2008;57:310-315

Performance of chromID ESBL, a chromogenic medium for detection of *Enterobacteriaceae* producing extended-spectrum *β*-lactamases

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(2) Service de Batériologie-Virologie, INSERM U914 : Emerging Resistance to Antibiotics, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris-Sud, Université Paris XI, 78 Rue de Général Leclerc, 94275 Le Kremlin- Bicêtre, France

The chromogenic agar medium chromID ESBL (bioMérieux) was compared with BLSE agar medium (AES) for selective isolation and presumptive identification of extended-spectrum ß-lactamase (ESBL)-producing *Enterobacteriaceae* from clinical samples.

A total of 765 samples (468 rectal swabs, 255 urine samples and 42 pulmonary aspirations) obtained from 547 patients was processed. All bacterial strains isolated on either medium were further characterized using biochemical tests, and ESBL producers were confirmed by synergy testing. Genetic characterization of ESBL genes was determined by PCR. A total of 33 ESBL-producing *Enterobacteriaceae* strains [*Escherichia coli* (n=16), *Klebsiella pneumoniae* (n=8), *Enterobacter* spp. (n=3), *Citrobacter* spp. (n=5) and Proteus mirabilis (n=1)] was recovered.

The sensitivity after 24 h incubation was 88 % for chromID ESBL and 85 % for BLSE agar. At 48 h, the sensitivity of chromID ESBL increased to 94 % and was higher than that obtained with BLSE agar. The positive predictive value at 24 h for chromID ESBL was 38.7 % [95 % confidence interval (95 % CI) 28.3-50.2 %)], which was significantly higher than that for BLSE agar [15.4 %, 95% CI 10.1-21.5 %]. On both media, false-positive results were mostly due to *Pseudomonas aeruginosa* and to *Enterobacteriaceae* overproducing chromosomal cephalosporinase (*Enterobacter* spp.) or a chromosomal penicillinase (*Klebsiella oxytoca*).

This study showed that chromID ESBL, a ready-to-use chromogenic selective medium, is sensitive and specific for rapid, presumptive identification of ESBL-producing *Enterobacteriaceae*. Its chromogenic properties and its selectivity are particularly useful in specimens containing resident associated flora.

KEY POINTS

→ chromID® ESBL is a reliable culture medium for screening and presumptive identification of ESBL producing *Enterobacteriaceae.*

A multi-center evaluation of 3 selective screening agars for the detection of extended-spectrum ß-lactamases

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INTRODUCTION

Enterobacteriaceae producing Extended-Spectrum β-Lactamases (ESBLs) are an emerging problem. This sustains the need for rapid and accurate screening methods, directly on stool samples or rectal swabs.

MATERIALS AND METHODS

Over a 4-month period (Nov 2010-Feb 2011), admission screening was performed at 2 Belgian hospitals: all patients admitted at the intensive care unit or at the geriatric ward were screened using a rectal swab (Eswab®, Copan). One hundred µl of the transport medium was inoculated onto 3 commercial screening agars: ChromID™ (bioMérieux), Brilliance™ agar (Oxoid) and BLSE agar (Chemunex). All agars were incubated overnight. If there was growth on at least 1 agar, identification and antimicrobial susceptibility testing was performed on the Microscan WalkAway® plus system (Microscan) (Siemens). Further confirmation of ESBL was performed using the double disk method and the ESBL plus® panel on the Microscan.

RESULTS

In total 504 samples were collected: 310 samples yielded no growth and 194 samples yielded growth on at least 1 agar. Overall 66 ESBL producing strains were found. One strain was missed with the ChromID™ agar and 8 strains were missed using the Brilliance™ agar. The study with the BLSE agar was prematurely stopped due to low sensitivity and specificity.

The prevalence of ESBL's was 14.7% at the intensive care unit in Brugge and was respectively 9.6% and 16.4% at the intensive care unit and at the geriatric ward in Knokke.

The sensitivity was 98.5% (ChromID™), 88.0% (Brilliance™ agar) and 83.7% (BLSE agar), respectively.

The specificity was equal for ChromID™ and Brilliance™ agar: 87% and was only 70% for the BLSE agar.

	ChromID™	Brilliance™ agar	BLSE agar
Total number of samples	N=504	N=504	N=425*
Growth	N= 120	N=114	N=153
ESBL positive samples	N=65	N=58	N=41
E. coli	54	50	36
E. aerogenes	4	3	1
K. oxytoca	3	2	1
K. pneumoniae	3	3	3
P. vulgaris	1	-	-

*The study with the BLSE agar was prematurely stopped due to low sensitivity and specificity.

CONCLUSION

There are no clear guidelines in literature on the size of inoculum for these screening agars.These agars are easy to use and give a first result after 24 h incubation. The ChromID™ and the Brilliance™ agar show similar performance in specificity, where as ChromID™ shows higher sensitivity compared to Brilliance™ agar. Further confirmation of ESBL remains necessary with both agars. The ESBL plus® panel contains a higher range of dilutions and is therefore much more sensitive to detect ESBL's compared to the conventional gram negative panel on the Microscan.

KEY POINTS

→ chromID® ESBL shows a significantly higher sensitivity for ESBL screening compared to Brilliance ESBL.

Evaluation of a chromogenic bioMérieux chromID™ESBL medium for screening and presumptive identification of extended-spectrum ß-lactamase-producing *Enterobacteriaceae* from surveillance cultures

C. Sánchez-Carrillo, M. Rivera, E. Cercenado, E. Bouza (Madrid, Spain)

OBJECTIVES

We evaluated a selective and differential chromogenic medium, chromID™ ESBL (bioMérieux, Marcy l'Etoile, France) which enables selective isolation and presumptive identification of extended spectrum ß-lactamase-producing *Enterobacteriaceae* (ESBL) from clinical samples.

METHODS

A total of 293 surveillance rectal swab samples were in parallel inoculated to chromID ESBL and MacConkey agar (MCK, Tec-Laim. Madrid. Spain) and incubated for 24-48h.

Pink-burgundy, green-blue and brown colonies on chromID ESBL were presumptively identified as ESBL-producing *Escherichia coli, Klebsiella/ Enterobacter/Serratia/Citrobacter* group or *Proteeae* respectively.

Any coloured colonies (pink-burgundy, green-blue or brown) and all oxidase-negative colonies on MCK were performed combined double disc method for ESBL confirmation and were also identified and tested susceptibility by the MicroScan system (Dade Behring).

RESULTS

Overall, 95 *Enterobacteriaceae* isolates were recovered on 91 specimens (31.1% of the total specimens studied) by at least one of the two media. Eighty-four specimens were positive by both media and eight specimens were positive only by chromID ESBL (7.7% of all positive samples).

The distribution of ESBL-producing microorganisms was as follows: 64 *E. coli*, 21 *Klebsiella pneumoniae*, 7 *Klebsiella oxytoca*, 1 *Citrobacter amanolaticus*, 1 *Enterobacter cloacae* and 1 *Enterobacter aerogenes*.

The overall sensitivity, specificity, positive predictive value and negative predictive value of chromID ESBL were: 100.0%, 92.6%, 85.8%, and 100.0%.

On chromID ESBL, a total of 18 non-ESBL-producing organisms presented the chromogenic appearance of ESBL-producers: *E. cloacae* (7), *E. coli* (4), *Klebsiella* spp. (2), *Pseudomonas aeruginosa* (2), *Serratia maecescens* (1) *Citrobacter freundii* (1), *Stenotrophomonas maltophilia* (1). Most of these false positive cephalosporinase were due to *Enterobacteriaceae* overproducing chromosomical cephalosporinase.

CONCLUSIONS

Our results showed that chromID ESBL is an appropriate medium for the screening and presumptive identification of ESBL-producing *Enterobacteriaceae* directly from surveillance specimens.

The enhanced recovery and the easier detection of ESBL-producing *Enterobacteriaceae* using this medium would benefit the patients and would decrease costs associated to infections caused by ESBL-producing microorganisms.

KEY POINTS

- → chromID® ESBL enables easy screening.
- → chromID® ESBL increased the recovery rate of ESBL-producing *Enterobacteriaceae* compared to conventional method.

Comparison of the chromID™ ESBL medium and MacConkey agar supplemented with ceftazidime (5mg/l) for the detection of extended-spectrum beta-lactamase producing *Enterobacteriaceae* from rectal swabs in hospitalized patients

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OBJECTIVES

Rapid identification of patients colonized with extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* is useful for the early detection and control of nosocomial outbreaks. The aim of this study was to evaluate the clinical diagnostic performance of the selective chromogenic medium chromID™ ESBL (bioMérieux, Marcy-l'Etoile, France), compared with our in-house medium CTAZ (MacConkey agar + ceftazidime 5 mg/l) for the detection of ESBL-producing *Enterobacteriaceae* from rectal swabs in ICU patients.

METHODS

From 5/11 to 17/12/2007, ICU hospitalized patients (n=299) were screened by sampling rectal swabs (n=436) using the Eswab (Copan, Italy).

After homogenization by vortexing for 15 s, 100 µl of the Eswab were inoculated onto chromID ESBL and CTAZ. Plates were incubated at 35°C and read after 18 and 48 h.

Identification and susceptibility testing were performed by using the VITEK® 2 system.

The presence of ESBL was confirmed by combined double disks according to CLSI guidelines.

Genotypic characterization was determined by PCR assays targeting bla_{TEM}, bla_{SHV} and bla_{CTX-M} genes.

Isolates harbouring bla_{TEM} and bla_{SHV} were further analyzed by sequencing to identify the ESBL.

A daily quality control was performed on CTAZ by using reference strains, *E. coli* ATCC 25922 (ESBL-negative) and *K. pneumoniae* ATCC 700603 (ESBL-positive), and according to CLSI guidelines for selective media.

RESULTS

One hundred and six specimens yielded growth on at least one of the selective media (Table 1) and 330 specimens were culture negative on both media.

Of 95 *Enterobacteriaceae* strains isolated from 48 patients (16.1%), 69 ESBL-positive strains were found in 32 patients (10.7%) (Table 1).

All *Enterobacteriaceae* strains produced the expected colour colonies on chromID ESBL, except for 4 *E. coli* isolates (colourless after 18 h, pink-burgundy after 48 h).

CTX-M derived enzymes (n=53) were the most frequently encountered ESBL, especially in *E. coli* and *E. cloacae*. They were significantly more frequently recovered on chromID ESBL than on CTAZ (p<0.0001) (Table 2).

TEM (n=10) and SHV (n=6) were found in *E. coli, K. pneumoniae* and *Enterobacter* spp. They were more frequently recovered on CTAZ than on chromID ESBL (p<0.0001).

Group/species	No. of isolates recovered on: Total ESBL	chromID	CTAZ media	Both
Escherichia coli	48	38	34	24
KESC group[a]: *Klebsiella oxytoca*	1	1	0	0
K. pneumoniae	10	8	10	8
Enterobacter aerogenes	6	2	6	2
E. cloacae	24	22	23	21
Citrobacter freundii	1	1	1	1
C. koseri	1	1	0	0
Hafnia alvei	1	0	1	1
PMP group[b]:				
Morganella morganii	2	0	2	0
Proteus vulgaris	1	1	0	0
Total no. of *Enterobacteriaceae*	95	74	77	56
Others *Pseudomonas aeruginosa*	24	16	19	11
Acinetobacter baumannii	5	5	0	0
NF-GNB	2	1	1	0
Gram + bacteria	6	5	1	0

a KESC: Klebsiella-Enterobacter-Serratia-Citrobacter group (including *H. alvei*)
b PMP: Proteus-Morganella-Providencia group

Table 2: *Distribution of ESBL-producing strains (n=69) detected by medium and ESBL class*

species (no. of isolates)	Classes of enzymzes (no. of isolates)	No. of isolates recovered on: chromID ESBL	CTAZ	Both media
E. coli (38)	TEM-40 (1)	0	1	1
	TEM-52 (6)	4	6	4
	TEM-78 (2)	0	2	0
	TEM-78 (2)	0	2	0
	CTX-M 1 (18)	18	11	11
	CTX-M 2 (9)	9	6	6
	CTX-M 9 (2)	2	1	1
E. cloacae (20)	CTX-M 9 (7)	7	6	6
	SHV-12 (3)	2	3	2
	CTX-M 9/SHV-12 (10)	9	10	9
K. pneumoniae (8)	CTX-M 1 (4)	4	4	4
	CTX-M 9 (1)	1	1	1
	SHV?[a] (3)	3	3	3
E. aerogenes (1)	TEM-24	1	1	1
K. oxytoca (1)	CTX-M 9	1	0	1
C. koseri (1)	CTX-M 1	1	0	1

A sequencing could not determine the subtype (probable presence of different subtypes)

Table 1: *Distribution of bacterial isolates (n=137) recovered from rectal swabs (n=106)*

The diagnostic performance of media was assessed by specimens, by bacterial isolates and *Enterobacteriaceae* isolates (Table 3). No significant difference was observed.

Table 3: *Performance of the selective media for detection of ESBL-positive Enterobacteriaceae (n=69)*

Medium	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)
Specimens (n=436)				
chromID ESBL	89.1	91.9	61.3	98.3
CTAZ	89.1	89.2	54.4	98.3
Organisms (n=137)				
chromID ESBL	89.9	33.8	57.9	76.7
CTAZ	81.2	36.8	56.6	65.8
Enterobacteriaceae (n=95)				
chromID ESBL	89.9	53.9	83.8	66.7
CTAZ	81.2	19.2	72.7	27.8

CONCLUSIONS

1. In our ICU department, chromID ESBL and CTAZ media demonstrated equivalent overall performance for detection of rectal carriage of ESBL-producing *Enterobacteriaceae*.
2. CTX-M positive isolates, which are the predominant ESBL, were more frequently recovered on chromID ESBL.
3. The main advantages of chromID ESBL were the direct identification of most *E. coli* strains and the cost-saving due to a lower number of specimens requiring identification of non-*Enterobacteriaceae* strains eventhough the cost of chromID ESBL is 4 times more than CTAZ (including preparation workload and quality control).

KEY POINTS

→ chromID® ESBL allows the direct identification of *E. coli.*

→ chromID® ESBL reduces the cost and workload.

chromID® VRE

chromID® VRE agar* is a selective chromogenic medium for the detection of *E. faecium* and *E. faecalis* showing acquired vancomycin resistance (VRE), in at-risk patients.

chromID® VRE agar enables the differentiation of *Enterococcus faecium* and *E. faecalis. E. faecium* and *E. faecalis* with acquired vancomycin resistance (mainly genotypes *van*A and *van*B) are multiresistant bacteria which can be responsible for healthcare-associated infections. The detection of this resistance is particularly important for the prevention and epidemiological surveillance of these infections and also to prevent the emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA), by transmission of the *van*A gene.

chromID® VRE agar (patents pending) consists of a rich nutritive base including a variety of peptones. It also contains two chromogenic substrates and a mixture of antibiotics including vancomycin (8 mg/l) which enable:

- the specific and selective growth of VRE.
- the direct detection and the differentiation of *E. faecium* and *E. faecalis* through the characteristic color of colonies:
  E. faecium: violet color for β-galactosidase-producing strains.
  E. faecalis: blue-green color for β-glucosidase producing strains.

The selective mixture inhibits:
- enterococci strains that do not express acquired vancomycin resistance,
- enterococci species that express natural vancomycin resistance (vanC genotype: *E. gallinarum* and *E. casseliflavus*), most Gram-negative and Gram-positive bacteria, yeasts and molds.

* See package insert for more information

CONTENTS

➔ ARTICLES

➔ POSTERS / ORAL PRESENTATION

CHINESE MEDICAL JOURNAL
2012;125:3450-3457

Extensive contact tracing and screening to control the spread of vancomycin-resistant Enterococcus faecium ST414 in Hong Kong

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(3) Department of Surgery (Chung HP), 4 Infection Control Team (Lo WK), Tung Wah Hospital, Hong Kong Special Administrative Region, China

The objective of this study was to communicate the control measures used to eradicate an outbreak of vancomycin-resistant enterococci (VRE) in a hospital network in Hong Kong, and demonstrate the effectiveness of extensive contact tracing and screening. The outbreak involved a University affiliated hospital and a convalescent hospital of 1600 and 550 beds respectively.

Computer-assisted analysis was utilized to facilitate contact tracing, followed by VRE screening using chromogenic agar (chromID VRE). A case-control study was conducted to identify the risk factors for nosocomial acquisition of VRE.

Eleven patients (1 exogenous case and 10 secondary cases) in two hospitals with VRE colonization were detected during our outbreak investigation and screening for 361 contact patients, resulting in a clinical attack rate of 2.8% (10/361). Case-control analysis demonstrated that VRE positive cases had a significantly longer cumulative length of stay ($P < 0.001$), a higher proportion with chronic cerebral and cardiopulmonary conditions ($P=0.001$), underlying malignancies ($P < 0.001$), and presence of urinary catheter ($P < 0.001$), wound or ulcer ($P < 0.001$), and a greater proportion of these patients were receiving β-lactam/β-lactamase inhibitors ($P=0.009$), carbapenem group ($P < 0.001$), fluoroquinolones ($P=0.003$), or vancomycin ($P=0.001$) when compared with the controls.

This study confirms the usefulness of extensive contact tracing and screening as part of a "search-and-confine" strategy as a successful tool for outbreak control in this healthcare region.

KEY POINTS

➔ VRE screening using chromID® VRE is an essential part of a successful outbreak control strategy.

DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE
2012;74:171–176

Performance of three chromogenic VRE screening agars, two Etest® vancomycin protocols, and different microdilution methods in detecting *van*B genotype *Enterococcus faecium* with varying vancomycin MICs

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Frequencies of *van*B-type *Enterococcus faecium* have increased in Europe in recent years. *Van*B enterococci show various levels of vancomycin MICs even below the susceptible breakpoint challenging reliable diagnostics.

This study investigated the performance of 3 chromogenic vancomycin-resistant enterococci (VRE) screening agars, 2 Etest® vancomycin protocols, and different microdilution methods to detect 129 clinical *van*B *E. faecium* strains.

One hundred and twelve (87%) *Van*B-type isolates were correctly identified as *Van*B-type Enterococcus by microdilution methods. The Etest® macromethod protocol was shown to be more sensitive than the standard protocol in identifying *van*B *E. faecium* strains, with sufficient specificity to correctly identify 15 *van*A/*van*B-negative strains. A few *van*B strains showed growth of microcolonies inside the Etest® vancomycin inhibition zones, suggesting a *van*B heteroresistance phenotype.

The three chromogenic VRE agars performed similarly with 121 (94%), 123 (95%), and 124 (96%) *van*B isolates that grew on Brilliance™ VRE Agar, CHROMagar™ VRE, and chromID™ VRE agar, respectively.

Using identical media and conditions, we did not identify different growth behaviour on agar and in broth.

KEY POINTS

➔ chromID® VRE shows better detection of *van*B *Enterococcus faecium* than other commercial agars.

KOREAN JOURNAL OF LABORATORY MEDICINE
2010;30:631-6

Evaluation of the Usefulness of Selective Chromogenic Agar Medium (ChromID VRE) and Multiplex PCR Method for the Detection of Vancomycin-resistant Enterococci.

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In this study, we evaluated the usefulness of a selective chromogenic agar medium and multiplex PCR for detection of VRE. Both of these techniques were compared with the conventional culture method for VRE detection.

The following 3 methods for detecting VRE infection in stool specimens were performed: the routine culture method, culturing in selective chromogenic agar medium (chromID VRE, bioMérieux, France), and multiplex PCR using the Seeplex VRE ACE Detection kit (Seegene Inc., Korea) with additional PCR for *van*C genes.

One hundred and nine VRE strains were isolated from 100 stool specimens by the routine culture method. With chromID VRE, all the isolates showed purple colonies, including *Enterococcus gallinarum* and *E. raffinosus*, which were later identified using the Vitek card. All VRE isolates were identified by the multiplex PCR method; 100 were *van*A-positive *E. faecium*, 8 were *van*A- and *van*C-1-positive *E. gallinarum*, and 1 was *van*A-positive *E. raffinosus*.

Accurate and early detection of vancomycin-resistant enterococci (VRE) is critical for controlling nosocomial infection. Culturing the isolates in chromID VRE after broth enrichment appears to be an accurate, rapid, and easy routine screening method for VRE surveillance. Multiplex PCR is relatively expensive and needs skilled techniques for detecting VRE, but it can be an auxiliary tool for rapid detection of genotype during a VRE outbreak.

KEY POINTS

➔ chromID® VRE is compatible with enrichment broth for rapid and accurate routine screening.

JOURNAL OF MICROBIOLOGICAL METHODS
2009;77:124-126

New selective and differential chromogenic agar medium, chromID™ VRE, for screening vancomycin-resistant *Enterococcus species*

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The aim of this study was to evaluate the usefulness of a novel differential culture media, chromID VRE agar, for the isolation of VRE in a clinical laboratory. In addition, chromID VRE was compared to bile esculin agar supplemented with 6 µg/ml vancomycin (BEV).

Ninety-six vancomycin-resistant and sensitive clinical enterococcal isolates (30 *E. faecalis*, 30 *E. faecium*, 11 *E. raffinosus*, 12 *E. casseliflavus* and 13 *E. gallinarum*) were used for testing. The *van*A, *van*B, and *van*C genes were detected by PCR. Inoculum of pure culture about 10^6 CFU/ml (8–10 single colonies) was streaked directly onto chromID VRE agar and examined after 24 h and 48 h incubation.

Thirty stool samples (15 from hospitalized VRE patients and 15 from healthy, community-based volunteers) were plated directly onto chromID VRE agar and BEV or after an overnight enrichment step (18–24 h) in BHI broth supplemented with 30 µg/ml vancomycin and incubated and examined as described above.

For stool samples, the sensitivity for detecting VRE for both media did not change significantly at 48 h of incubation ($p<0.05$), but was significantly higher after the enrichment step. For direct culture, the specificity of chromID VRE was significantly higher compared to BEV (100% vs 60%) after 24 h of incubation.

The results obtained in the present study indicate that chromID VRE agar also easily detected dual colonization with VR *E. faecium* and VR *E. faecalis* strains. In addition, chromID VRE revealed good abilities to isolate and identify VR *E. faecium* and *E. faecalis* strains of both VanA and VanB phenotypes, as well as successfully detecting acquired vancomycin resistance present in *E. casseliflavus*, *E. gallinarum* and *E. raffinosus*.

In conclusion, chromID VRE agar is an easy-to-use, cost-and time-effective medium for the isolation of VRE and the differentiation of VR *E. faecium* and VR *E. faecalis* strains. Compared to BEV medium, there is no need for confirmatory tests to identify phenotype resistance and *E. faecalis* isolates.

KEY POINTS

- → For direct culture the specificity of chromID® VRE is significantly higher compared to bile Esculin agar (100% versus 60%) after 24 hours of incubation.
- → chromID® VRE easily detects dual colonization with vancomycin-resistant *E. faecium* and vancomycin-resistant *E. faecalis*.
- → chromID® VRE enables to isolate and identify vancomycin-resistant *E. faecium* and *E. faecalis* strains of both VanA and VanB phenotypes.

Evaluation of bioMérieux chromID™ VRE Agar for the detection of Vancomycin Resistant Enterococci (VRE)

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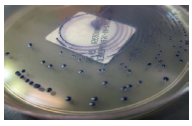
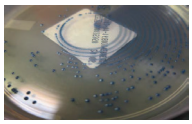
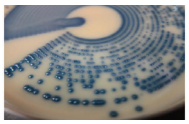
INTRODUCTION

- Evaluation of new formulation of the bioMérieux chromID™ VRE chromogenic agar
- Study Objective: Compare sensitivity, specificity, PPV, NPV and selectivity (breakthrough) by evaluating two chromogenic agars: bioMérieux chromID™ VRE, Oxoid® Brilliance VRE
- Current process: Oxoid® Brilliance VRE agar ➔ Molecular confirmation

Table 1. VRE Media Specifications

Media	bioMérieux chromID™ VRE	Oxoid® Brilliance VRE
Incubation Time	24 - 48 hours	24 hours
E. faecalis	Blue-green	Light blue - blue
E. faecium	Violet	Pink, purple, purple-pink or dark blue
Confirmatory tests	Gram stain	

Figure 1. VRE Chromogenic Agars

	bioMérieux chromID™ VRE	Oxoid® Brilliance VRE
VRE faecium		
VRE faecalis		

STUDY METHOD

- 500 samples – 323 Rectal swabs (screening), 176 Stool specimens with a *C. difficile* toxin test request, 1 Wound swab
- Samples ➔ 2.0 mL saline ➔ vortexed ➔ PREVI Isola (0.1 mL) streaking on both media (incubated at 35°C aerobically)
- All media examined after 1 and 2 days incubation
- Confirmatory real-time PCR (in-house developed, internally controlled) for *van*A and *van*B on "positive" cultures

Biochemical ID & Susceptibility Testing

- Positive colonies were subcultured onto Columbia blood agar
- Biochemical Identification –Gram stain, Vitek 2 GP card
- Vancomycin MIC Susceptibility Testing – Etest on Mueller Hinton Agar

Molecular Confirmation

- Any colony of the expected colour

➔ 100 µL of lysis solution

➔ Vortexed, heated for 20 minutes at 95°C, then centrifuged

➔ 2 µL of lysed sample ➔ PCR

RESULTS

- Positive sample = positive by at least one culture method and confirmed positive by PCR
- Negative sample = negative in ALL culture methods or did not contain the *van*A or *van*B genes as detected by PCR
- 500 samples tested ➔ 43 were confirmed positive for *van*A
  ➔ 457 samples were negative
- 43 of 43 positive samples were positive on both plates
- Negative samples were either no growth, showed "negative" colony morphologies/biochemical ID tests, or VRE was ruled out by PCR.

Sample Workflow

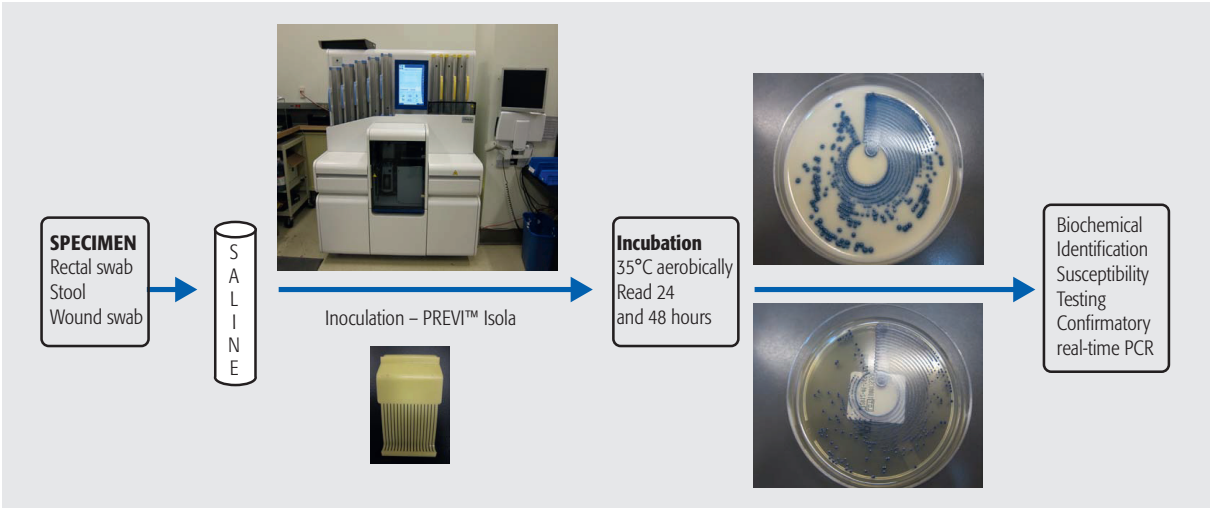


Table 2. Number of vanA or vanB positive isolates recovered by each medium (Total Positive =43)

	bioMérieux chromID™ VRE	Oxoid® Brilliance VRE
VRE (*van*A)	43	43
VRE (*van*B)	0	0

Table 3. Sensitivity,Specificity, PPV, NPV, Selectivity

	bioMérieux chromID™ VRE		Oxoid® Brilliance VRE	
Day	1	2	1	2
Sensitivity	88.4%	100%	88.4%	100%
Specificity	99.6%	98.9%	99.6%	98.1%
PPV	95%	89.6%	95%	82.7%
NPV	98.9%	100%	98.9%	100%
Selectivity - # (Breakthrough)	5	6	21	92

Shaded boxes are the manufacturers' recommended incubation and reading time.

DISCUSSION

Sensitivity:

- Similar, with improvement by an additional 24h incubation.

	bioMérieux chromID™ VRE		Oxoid® Brilliance VRE	
Day	1	2	1	2
Sensitivity	88.4%	100%	88.4%	100%

Shaded boxes are the manufacturers' recommended incubation and reading time.

Specificity:

- Similar
- Additional incubation time (>24 hours) slightly compromised specificity as breakthrough of non-VRE organisms was observed
- Need to have a confirmatory test for VRE (PHC ➔ confirmatory in-house PCR test targeting the *van*A and *van*B genes)

	bioMérieux chromID™ VRE		Oxoid® Brilliance VRE	
Day	1	2	1	2
Specificity	99.6%	98.9%	99.6%	98.1%

Shaded boxes are the manufacturers' recommended incubation and reading time.

Selectivity:

- The bioMérieux chromID™ VRE exhibited less breakthrough of non-chromogenic colonies (clear or white)

	bioMérieux chromID™ VRE		Oxoid® Brilliance VRE	
Day	1	2	1	2
PPV	95%	89.6%	95%	82.7%
NPV	98.9%	100%	98.9%	100%
Selectivity -# (Breakthrough)	5	6	21	92

Shaded boxes are the manufacturers' recommended incubation and reading time.

CONCLUSION

- The new formulation of bioMérieux chromID™ VRE exhibited similar sensitivity and specificity, albeit with less breakthrough of non-chromogenic colonies (clear or white), compared to Oxoid® Brilliance VRE
- For optimized VRE recovery, an additional reading at 48 hours is recommended for both media

KEY POINTS

➔ The study shows the need for 48 hour incubation with Oxoid Brilliance VRE to optimize VRE recovery.

➔ chromID® VRE has a higher selectivity with less breakthrough compared to Oxoid Brilliance VRE.

Effectiveness of chromID™ VRE medium for detection of *Enterococcus faecalis* and *Enterococcus faecium* showing acquired vancomycin resistance

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INTRODUCTION

Enterococci, although normally present in the intestinal and female genital tracts of humans, may cause infections in other parts of the body such as urinary tract, bloodstream or from wounds. Vancomycin is a glycopeptide antibiotic often used to treat Gram-positive infections such as these. Since the late 1980's, Vancomycin-Resistant *Enterococcus* (VRE) has emerged as an important pathogen and is now the third most common organism associated with nosocomial (health-care associated) infections. The Centers for Disease Control and Prevention (CDC) reports that during 2006 and 2007 enterococci caused about 1 of every 8 infections in hospitals and about 30% of these are VRE. Rapid identification of VRE will affect patient treatment, prevent transmission and control outbreaks. bioMérieux, Inc. developed a chromogenic medium for the screening of Vancomycin resistance (VRE) in at risk patients. The purpose of this study was to demonstrate the safety and effectiveness of chromID VRE as used in a clinical setting.

METHODS

Redundant stool specimens were inoculated to Bile Esculin Azide Agar with Vancomycin (BEAV) and chromID VRE. Both agar plates were incubated aerobically at 35°C and the chromID VRE was incubated in the dark. Plates were examined at 24 and 48 hours for the characteristic presence of light brown to black colonies surrounded by dark zones on BEAV, and blue-to-green color colonies for VRE-faecalis and violet color colonies for VRE-faecium on chromID VRE agar plates.

Figure 1 shows the testing algorithm utilized for this study. Identification of *Enterococcus faecalis* and *Enterococcus faecium* was confirmed by Gram stain, catalase and the VITEK 2 GP ID Card. If applicable, supplemental testing was performed to confirm identification. Vancomycin resistance was confirmed by agar dilution and van gene PCR testing.

Quality control isolates (20 replicates of *Enterococcus faecalis*, ATCC 29212, *Enterococcus faecalis*, ATCC 51299, *Enterococcus faecium*, CCUG 36804 and *Enterococcus faecium*, ATCC 700221), reproducibility isolates (10 well characterized strains) and 406 fresh clinical stool specimens were tested. Reproducibility tests were performed in triplicate daily for three days. The sensitivity/specificity and positive/negative predictive values (PPV/NPV) were calculated for detection of VRE on chromID VRE and BEAV at 24 and 48 hours.

RESULTS

Of the 100 VRE positive samples detected, 10 were VRE-*faecalis* and 99 were VRE-*faecium* (9 samples detected both VRE-*faecalis* & VRE-*faecium*). All 10 VRE-*faecalis* were detected at 24 hours.

Conventional Test Methods (based on results from either test medium):
ID = *E. faecalis* or *E. faecium*
Vancomycin agar dilution = Resistant
Van gene PCR = *van*A or *van*B

The sensitivity and specificity for detection of VRE compared to conventional identification and susceptibility testing for chromID VRE was 96.9% and 100% respectively at 24 hours, and 97% and 100% respectively at 48 hours, while the PPV/NPV was 100% and 99.0% respectively at both 24 & 48 hour. The sensitivity and specificity for detection of VRE compared to BEAV for chromID VRE was 96.8% and 98.7% respectively at 24 hours, and 96.9% and 99.4% respectively at 48 hours. The PPV/NPV was 95.8% / 99% at 24 hours & 97.9% / 99% at 48 hours. Overall agreement for chromID VRE quality control and reproducibility was 100% (see tables 1-6).

Table 1. *Overall Percent Agreement of chromID VRE vs Conventional Test Methods at 24 Hours*

	Conventional Pos	Conventional Neg	TOTAL
chromID VRE Pos	95	0	95
chromID VRE Neg	3	308	311
TOTAL	98	308	406

Sensitivity= 96.9%, Specificity=100%, Positive Predictive Value=100%, Negative Predictive Value=99%

Table 2. *Overall Percent Agreement of BEAV vs Conventional Test Methods at 24 Hours*

	Conventional Pos	Conventional Neg	TOTAL
BEAV	94	0	94
BEAV	4	308	312
TOTAL	98	308	406

Sensitivity= 95.9%, Specificity=100%, Positive Predictive Value=100%, Negative Predictive Value=98.7%

Table 3. *Overall Percent Agreement of chromID VRE vs Conventional Test Methods at 48 Hours*

	Conventional Pos	Conventional Neg	TOTAL
chromID VRE Pos	97	0	97
chromID VRE Neg	3	306	309
TOTAL	100	306	406

Sensitivity= 97.0%, Specificity=100%, Positive Predictive Value=100%, Negative Predictive Value=99%

Table 4. *Overall Percent Agreement of BEAV vs Conventional Test Methods at 48 Hours*

	Conventional Pos	Conventional Neg	TOTAL
BEAV	98	0	98
BEAV	2	306	308
TOTAL	100	306	406

Sensitivity= 96.9%, Specificity=100%, Positive Predictive Value=100%, Negative Predictive Value=99%

Table 5. *Overall Percent Agreement of chromID VRE vs BEAV at 24 Hours*

	BEAV Pos	BEAV Neg	TOTAL
chromID VRE Pos	91	4	95
chromID VRE Neg	3	308	311
TOTAL	94	312	406

Sensitivity= 96.8%, Specificity=98.7%, Positive Predictive Value=95.8%, Negative Predictive Value=99%

Table 6. *Overall Percent Agreement of chromID VRE vs BEAV at 48 Hours*

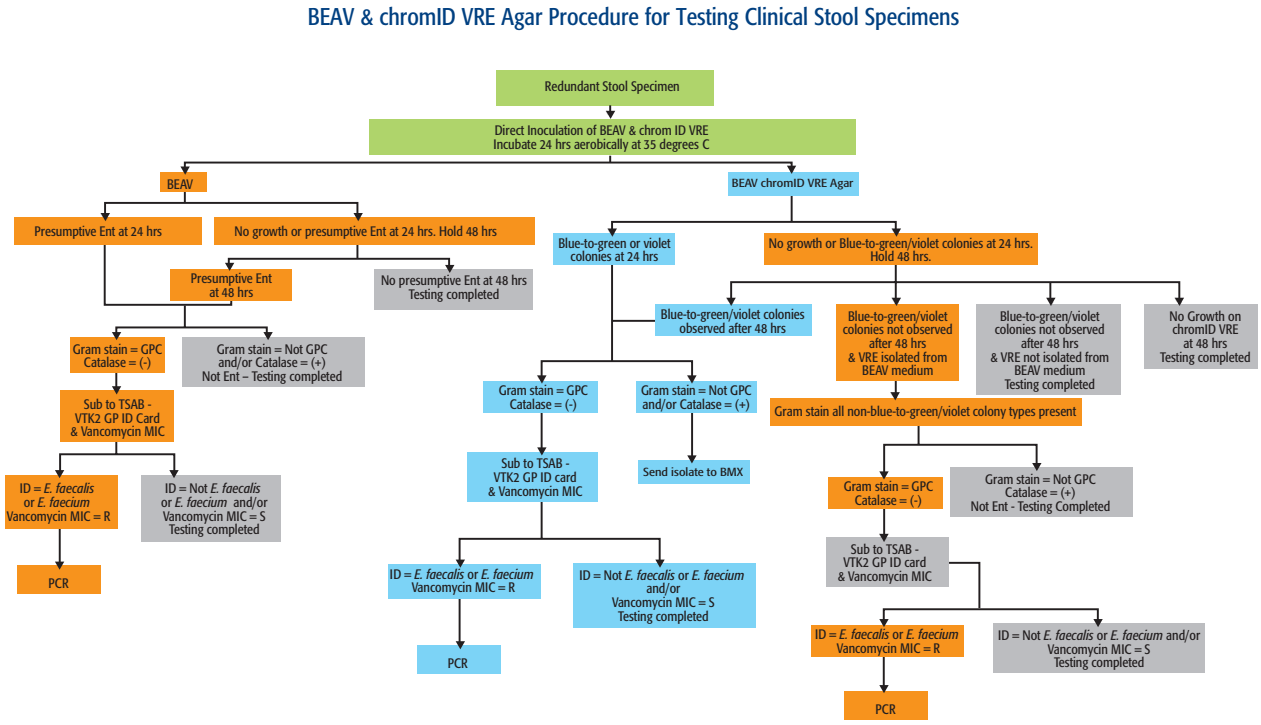
	BEAV Pos	BEAV Neg	TOTAL
chromID VRE Pos	91	4	95
chromID VRE Neg	3	308	311
TOTAL	94	312	406

Sensitivity= 96.9%, Specificity=98.7%, Positive Predictive Value=95.8%, Negative Predictive Value=99%

CONCLUSION

This evaluation provides convincing evidence that the performance of chromID VRE is an excellent screening method with stool specimens for detecting Vancomycin resistant *Enterococcus faecium* and *Enterococcus faecalis* in the clinical laboratory and can provide accurate results within 24 - 48 hours (95% of VRE positive samples were detected at 24 hours).

Figure 1. *Testing Procedures for chromID VRE and BEAV AGAR from Clinical Stool Specimens.*



KEY POINTS

→ chromID® VRE reduces the turn-around time : 95 % of VRE positive sample were detected at 24 hours.

→ chromID® VRE enables identification of vancomycin-resistant *E. faecium* and *E. faecalis* as well dual colonization.

chromID™ VRE to screen for Vancomycin-Resistant *Enterococcus faecium* and *Enterococcus faecalis* in stool specimens

Tara Reimer[1], Katherine Riebe[1], and Nathan A Ledeboer[1, 2]

(1) Dynacare Laboratories and (2) Department of Pathology, Medical College of Wisconsin, Milwaukee, Wisconsin

INTRODUCTION

Enterococcus species are members of the normal intestinal flora, and are the most common aerobic Gram-positive cocci found in the large bowel. The organisms have gained notoriety, however, as nosocomial pathogens, and are now grouped as the third most common blood-borne pathogen in the United States. At least one factor accounting for this pathogenicity is their intrinsic resistance to a variety of antimicrobial agents, including cephalosporins, co-trimoxazole, clindamycin, and beta-lactamase resistant penicillins. However, an even more alarming pattern arose in 1988, as plasmid-mediated resistance to vancomycin was first described. The most common acquired resistance-conferring enzyme is *van*A, which is a transposon-mediated, inducible gene conferring high-level resistance to vancomycin and teicoplanin, and can be located on plasmid or chromosomal DNA. Glycopeptide resistance mediated by *van*B is less common, and confers resistance to vancomycin, but not teicoplanin. Less common determinants conferring vancomycin resistance include *van*D, *van*E, *van*F, and *van*G, which encode moderate to low-level resistance to glycopeptides.

The clinical impact of VRE has been examined in several studies, with the most notable consequences being increased mortality, increased lengths of hospital stay, as well as increased costs of hospitalization associated with VRE infection. For example, one retrospective case-control study demonstrated a 6% increase in mortality, and an average of 6-day increased length of hospitalization in patients infected or colonized with VRE, in comparison to controls without documented VRE involvement.

Recognizing the impact of VRE, the Hospital Infection Control Practices Advisory Committee (HICPAC) of the Centers for Disease Control and Prevention (CDC) in 1995 created recommendations to curb the epidemic of VRE infection in hospital settings. In addition to addressing healthcare workers as vectors for transmission, and recommending judicious use of antibiotics such as vancomycin, HICPAC recommended protocols for the early detection of VRE and precautionary isolation of involved patients.

This poster describes the use of a culture-based VRE detection method that allows rapid detection not allowed by conventional culture methods, but conveys similar benefits to traditional culture methods in regard to sensitivity, specificity, and potential for susceptibility and epidemiological studies.

MATERIALS AND METHODS

• *Study enrollment and collection of clinical specimens*

Stool specimens were submitted to Dynacare Laboratories, a reference laboratory with outreach in Wisconsin and northern Illinois. Patients were considered for inclusion in the study if they had fecal specimens submitted for VRE detection or *Clostridium difficile* EIA. These criteria were chosen to select a patient population at high risk for VRE colonization. Specimens submitted for this study were limited to fecal specimens collected in sterile containers. Collected specimens were transported and stored at 4°C up to 5 d before being plated on the control or experimental medium. This study was approved by the Medical College of Wisconsin Institutional Review Board.

• *Media and culture conditions*

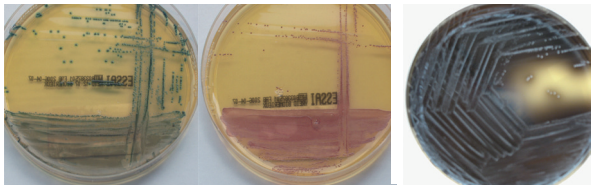
We compared the performance of the experimental chromogenic medium (chromID VRE; bioMérieux) with bile esculin azide agar supplemented with 6 µg/mL vancomycin (BEAV; Remel, Lenexa, KS). chromID VRE and BEAV plates were directly inoculated by submerging a sterile swab into the specimen, plating to the first quadrant, and streaked using the quadrant method. Plates were incubated at 35°C and examined for growth at 24 and 48 h time points. chromID plates with purple or green colonies were presumed positive for *E. faecium* or *E. faecalis*, respectively. BEAV plates with brown to black colonies were presumed positive for VRE. Following observation of the colony morphologies, individual unique colonies were subcultured onto Tryptic soy blood agar plates (Remel) for confirmatory identification and antimicrobial susceptibility testing. Identification of typically colored isolates was completed by Gram stain, catalase, PYR, and using the VITEK 2 automated identification system (bioMérieux). Susceptibility testing was performed using agar dilution as described by the Clinical Laboratory Standards Institute.

• *Multiplex PCR for detection of glycopeptide resistance genes and identification of E. faecium and E. faecalis.*

DNA was extracted per previously published methods. Multiplex PCR was adapted from the method previously described by Depardieu *et al.* (J. Clin. Microbiol. 2004, 43, 3390-3397). Amplified products were detected and quantified by electrophoresis on a 2100 BioAnalyzer system (Agilent, Massy, France) according to the manufacturer's instructions.

Figure 1 *- chromID VRE medium plated with E. faecalis or E. faecium and Bile Esculin Azide agar plated with E. faecium. Figures 1A and 1B demonstrate ability of chromID VRE to identify E. faecium and E. faecalis based on color of the colony. On chromID VRE, colonies of E. faecalis will appear blue-green and colonies of E. faecium will appear purple. For purposes of this study, fecal specimens were directly plated to chromID VRE and BEAV and observed for growth at 24 and 48 hours.*

Figure 1A *Figure 1B* *Figure 1C*



RESULTS

505 stool samples were plated on each test medium and examined after 24 and 48 hours of incubation.

Results are shown in Figures 2, 3 and 4 and Table 1.

Figure 2 *- Results from sensitivity analysis of chromID VRE and BEAV at 24 and 48 hours. Figure 2 demonstrates sensitivity of each medium for identification of VRE (E. faecalis or E. faecium).*

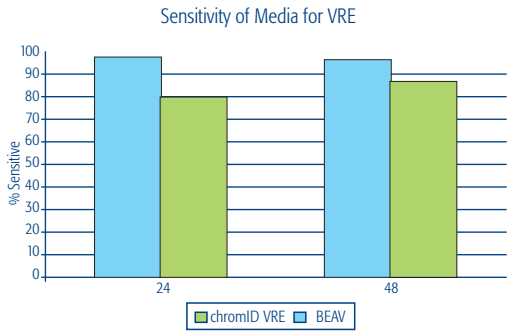


Figure 3 *- Specificity of chromID VRE and BEAV for VRE after 24 and 48 h.*

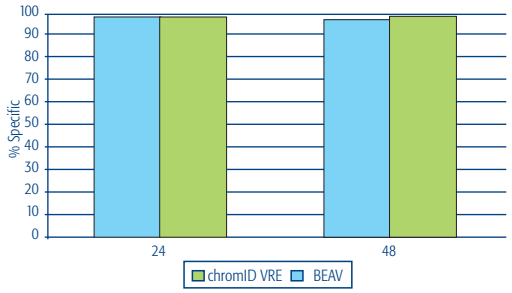


Figure 4*. Positive Predictive Value and Negative Predictive Value of chromID VRE and BEAV for VRE after 24 and 48 h.*

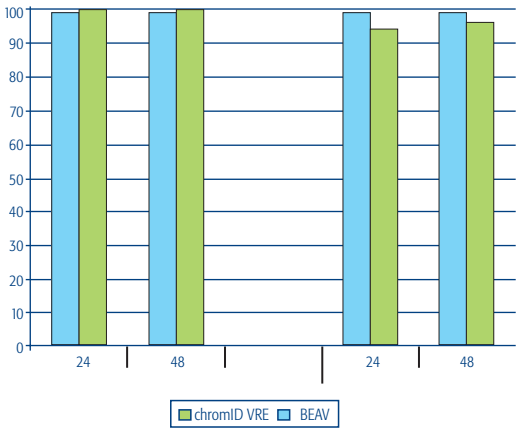


Table 1. *chromID VRE Clinical Performance Data. Performance of chromID VRE from all clinical trial sites compared to BEAV, Vancomycin MIC, and detection of vancomycin resistance determinants by PCR.*

chromID VRE vs BEAV

	24 Hours		48 Hours	
	VRE	Non-VRE	VRE	Non-VRE
chromID VRE	97	99.7	96.8	99.7
BEAV	86.9	100	91.6	100
chromID VRE vs Vancomycin MIC	99.4	98.5	99.4	99.6
chromID VRE vs van PCR	99.7	98.7	99.7	99.8

CONCLUSIONS

- chromID VRE represents the first chromogenic medium to be submitted to the United States Food and Drug Administration to identify VRE and differentiate *E. faecium* from *E. faecalis* in stool specimens.
- chromID VRE offers improved sensitivity versus BEAV in isolating and identifying VRE in feces.
  - BEAV provides identification of VRE, chromID VRE provides species level identification of *E. faecalis* and *E. faecium* for improved tracking of nosocomial epidemics.
  - Incubation for 48 h does not significantly improve sensitivity of either chromID VRE or BEAV.
- Specificity of BEAV and chromID VRE are not significantly different.
- Growth of contaminants that may be interpreted as false positives is slightly greater on BEAV versus chromID VRE.
  - Presence of contaminants potentially causing false positives significantly increases at 48 h on both media types.
- Unlike chromID VRE, BEAV promotes growth of vancomycin intermediate enterococci.
- chromID VRE is more likely to predict positive and negative VRE colonization versus BEAV.
- chromID VRE detected 23 more VRE pos samples at 24 h and 15 more at 48 h versus BEAV. BEAV detected 3 more VRE pos samples at 24 h, and 4 at 48 h that chromID VRE falsely called negative.

KEY POINTS

→ chromID® VRE offers improved sensitivity versus Bile Esculin Azide agar supplemented with Vancomycin (BEAV) in isolating and identifying VRE in feces.

→ chromID® VRE provides species identification of *E. faecalis* and *E. faecium* for improved tracking of nosocomial epidemics.

chromID® C. difficile

chromID® C. difficile agar* is a selective chromogenic medium for the detection and identification of *Clostridium difficile* in human specimens (stools of symptomatic patients).

The medium contributes to the diagnosis and epidemiological monitoring of *Clostridium difficile* infections.
Clostridium difficile is a causative agent of pseudomembranous colitis and more generally of healthcare-associated or antibiotic-associated diarrhea.
chromID® C. difficile agar consists of a rich nutritive base combining different peptones and taurocholate which favors the germination of spores. It contains a chromogenic substrate (patent pending) and a mixture of antibiotics which enable:

- the detection and identification of ß-glucosidase-producing *Clostridium difficile* strains based on the typical grey to black color of colonies,
- the inhibition of most Gram-positive and Gram-negative bacteria, yeasts and molds.

* See package insert for more information

CONTENTS

➔ ARTICLES

➔ POSTERS / ORAL PRESENTATION

JOURNAL OF MEDICAL MICROBIOLOGY
2013;83:164-166

Isolation of Clostridium difficile from faecal specimens – a comparison of ChromID C. difficile agar and cycloserine cefoxitin fructose agar.

Carson KC[1], Boseiwaqa LV[2], Thean SK[1], Foster NF[2] and Riley TV[2]
(1) PathWest Laboratory Medicine WA; (2) The University of Western Australia

The culture of toxigenic *Clostridium difficile* from stool specimens is still seen as the gold standard for the laboratory diagnosis of *C. difficile* infection (CDI). bioMérieux have released ChromID Cdiff (CDIF) chromogenic agar for the isolation and identification of *C. difficile* in 24 h. In this study, we compared CDIF to pre-reduced cycloserine-cefoxitin-fructose agar with sodium taurocholate (TCCFA) for the examination of glutamate dehydrogenase (GDH)-positive faecal specimens that were either GeneOhm positive or negative, using direct culture or culture following alcohol shock.

Direct culture on CDIF had a sensitivity of 100% and recovery of 94% while for TCCFA these were 87% and 82%, respectively. For GeneOhm positive alcohol shocked faecal samples, sensitivity and recovery on CDIF was similar to direct culture while on TCCFA they were about 10% higher. For direct culture, there was a significant difference between growth on CDIF at 24 h and TCCFA at 48 h ($p = 0.001$) and between the two media at 48 h ($p < 0.001$). A total of 142 strains of C. difficile were recovered from all GeneOhm positive samples used in this study and 11 (7.7%) of these were A-B-CDT- and may represent mixed infections of toxigenic and non-toxigenic *C. difficile*. The most dominant ribotype was UK 014 (14.7%) followed by 002 (11.9%) and 020 (11.9%), and 36% of toxigenic isolates, including an A-B+CDT- strain could not be assigned a UK ribotype.

CDIF outperforms pre-reduced TCCFA by negating the need for alcohol shock treatment and by giving a time saving of 24 h in the isolation of C. difficile. CDIF plates are also more selective than TCCFA and *C. difficile* colonies are easy to identify and sub-culture prior to strain typing.

KEY POINTS

- → chromID® C.difficile shows positive culture in 24 hours.
- → Colonies of *C.difficile* are easier to identify with chromID® C.difficile compared to conventional culture media.

JOURNAL OF CLINICAL MICROBIOLOGY
2013;51:1002-1004

Evaluation of the chromogenic agar chromID® C. difficile

Eckert C[1,3]; Burghoffer B[1,3]; Lalande V[2,3]; Barbut F[1,2,3]

(1) National Reference Laboratory for C. difficile, Hôpital Saint-Antoine, Assistance Publique-Hôpitaux de Paris, Paris, France
(2) Microbiology Unit, Assistance Publique-Hôpitaux de Paris, Hôpital Saint-Antoine, Paris, France.
(3) UPMC Univ Paris 06, GRC n°2, Epidiff, Paris, France

This study evaluated the performance of three selective media (chromID® C. difficile agar, TCCA (home-made) and the CLO medium) on 406 stool samples of patients suspected of having *C. difficile* infection.

Sensitivity of chromID® C. difficile agar 24h and 48h, CLO and TCCA was 74.1%, 87%, 85.2% and 70.4% respectively.

In contrast to the findings of Perry et al. (JCM 2010;48:3852-58), who do not recommend incubating plates beyond 24 hours, this study found that prolonging incubation to 48 hours, in cases of a negative result at 24 hours, enhances recovery of *C. difficile* strains on chromID C. difficile agar. However, this extra sensitivity should be weighed against extra turnaround time and indirect costs.

Identification of *C. difficile* on chromID® C. difficile agar in 24 hours is easy due to the colour of the colonies and its selectivity.

KEY POINTS

→ chromID® C.difficile allows direct identification of *C.difficile* in 24 hours due to characteristic coloration.

→ Sensitivity increases with an additional 24 hours' incubation.

JOURNAL OF HOSPITAL INFECTION
2013;83:164-166

Comparison of two selective media for the recovery of Clostridium difficile from environmental surfaces

Hill, KA; Collins, J; Wilson, L; Perry, JD; Gould, FK.
Microbiology Department, Freeman Hospital, Newcastle upon Tyne, UK

This study aimed to compare two culture media for their ability to isolate *Clostridium difficile* from environmental sites within a UK hospital. The media were cefoxitine-cycloserine egg yolk agar plus lysozyme (CCEY/L) and chromID C. difficile.

Four hundred and ninety-six environmental samples were taken from a wide range of environmental surfaces using sterile sponges (Polywipes) and were inoculated on to both media. *C. difficile* was recovered from 105 of 496 sites (21%) using a combination of both media. Fifteen samples were positive for *C. difficile* on both media, 77 were positive on chromID C. difficile only and a further 13 produced growth on CCEY/L only. The sensitivity of chromID C. difficile was 87.6% compared with 26.6% for CCEY/L ($P < 0.0001$). chromID C. difficile performed significantly better than CCEY/L for the recovery of *C. difficile* from the environment.

The significantly higher isolation rate of C. difficile on chromID C. difficile in this study supports the hypothesis that chromID C. difficile may be an effective medium for recovery of *C. difficile* spores in the environment. With its higher rate of recovery of *C. difficile*, and lower levels of non- *C. difficile* growth, the performance of chromID C. difficile was significantly better than CCEY/L for the recovery of *C. difficile* from environmental surfaces using the method described ($P < 0.0001$).

In conclusion, chromID C. difficile can be recommended for environmental screening, although further studies in different geographical areas would be beneficial to support these findings.

KEY POINTS

→ chromID® C.difficile can be used for the recovery of *C.difficile* spores in the environment and shows a higher sensitivity than current conventional culture media.

JOURNAL OF CLINICAL MICROBIOLOGY
2010;48:3852-8

Evaluation of a chromogenic culture medium for isolation of Clostridium difficile within 24 hours.

Perry JD[1], Asir K[1], Halimi D[2], Orenga S[2], Dale J[1], Payne M[1], Carlton R[1], Evans J[3], Gould FK[1]

(1) Department of Microbiology, Freeman Hospital, Newcastle upon Tyne, United Kingdom,
(2) Research & Development Microbiology, bioMérieux, La Balme-les-Grottes, France,
(3) Health Protection Agency, Newcastle upon Tyne, United Kingdom3

Rapid, effective methods for the isolation of *Clostridium difficile* from stool samples are useful to obtain isolates for typing or to facilitate accurate diagnosis of *C. difficile*-associated diarrhea. The aim of the present study was to evaluate a novel prototype chromogenic medium (ID C. difficile prototype [IDCd]) in comparison to commercially available media for the detection of C. difficile.

The chromogenic medium was compared using (i) 368 untreated stool samples also inoculated onto CLO medium, (ii) 339 stool samples subjected to alcohol shock and also inoculated onto five distinct selective agars, and (iii) standardized suspensions of 10 *C. difficile* ribotypes (untreated and alcohol treated) that were also inoculated onto five distinct selective agars.

Two hundred thirty-six isolates of *C. difficile* were recovered from 368 untreated stool samples, and all but 1 of these strains (99.6%) were recovered on IDCd within 24 h vs. 74.6% of isolates recovered on CLO medium after 48 h. Of 339 alcohol-treated stool samples cultured onto IDCd and five other selective agars, *C. difficile* was recovered from 218 samples using a combination of all media. The use of IDCd allowed recovery of 96.3% of isolates within 24 h, whereas 51 to 83% of isolates were recovered within 24 h using the five other media. Finally, when challenged with pure cultures, all 10 ribotypes of C. difficile generated higher colony counts on IDCd irrespective of alcohol pretreatment or duration of incubation.

In conclusion, IDCd is an effective medium for isolation of *C. difficile* within only 24 h with or without the use of alcohol-shock treatment. The main advantage of the chromogenic reaction was the formation of black colonies that contrasted sharply with the clear background agar, enabling easy detection of C. difficile (Fig. 1).

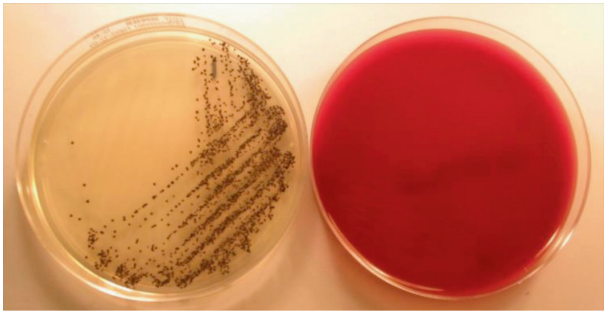


Fig. 1: Culture of C. difficile from a stool sample after 24 h of incubation on IDCd medium (left) and CLO medium (right). On IDCd, C. difficile forms typical black colonies, whereas the CLO medium plate shows no growth.

KEY POINTS

→ Easy detection of *C.difficile* on chromID® C.difficile due to the black coloration of the colonies whatever the specimen type: stool, stool after alcohol shock or suspensions of *C.difficile*.

"Black colonies": the new faster detection for *C. difficile* by culture

L. Prendergast

Melbourne Pathology, Melbourne, Australia

INTRODUCTION

Clostridium difficile can cause pseudomembranous colitis and other *C.difficile*–associated diseases.

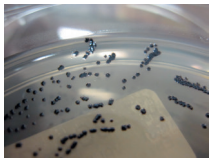
- The major cause of antibiotic-associated diarrhoea
- Recent cause of large hospital-acquired outbreaks.
- Recent outbreaks unusual in the severity and high mortality
- Recent outbreaks in Nth America and Europe predominantly due to a *Clostridium difficile* BI/NAP1/027, or ribotype 027.

Risk factors

- Age >65
- Antibiotics
  Broad spectrum cephalosporins, clindamycin and fluoroquinolones are the most frequently implicated antibiotics.
- Chemotherapy
- Proton-pump inhibitors

MATERIALS AND METHODS

CDIF Chrom ID™ *VS.* CC-BHIA +Tauro

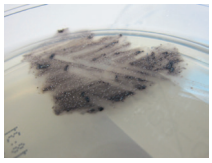


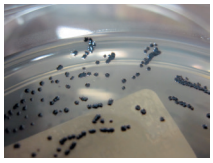
CDIF Chrom ID™ Culture positive at 20-24 hours

- Black colonies
- Typical odour
- Spreading colony morphology
- Confirm with gram stain

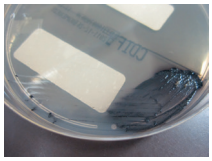
Cultures read at 20-24 hours as per manufacturer's instructions

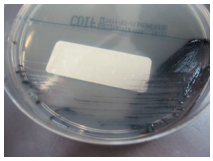
Negative and positive cultures at 20 hours



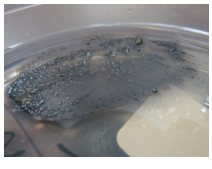


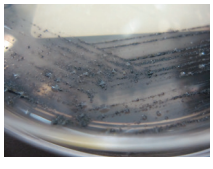
Negative cultures at 24 hours



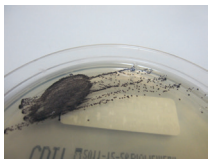


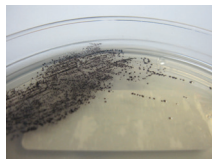
Negative cultures at 48 hours



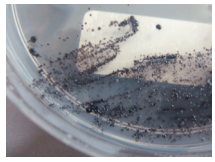


Positive cultures at 20-24 hours





Positive cultures at 48 hours



RESULTS

Total of 281 faeces samples plated onto CC-BHIA + Tauro (Oxoid) and CDIF ChromID™ (bio Merieux)

- Negative on both media 224
- Positive on both media 53
- Pos CC-BHIA, Neg CDIF ..... 3 *
- Neg CC-BHIA, Pos CDIF 1

* one plate 4 colonies, one plate 1 colony only and 3rd plate 5 colonies

Moxifloxacin susceptibility results

- 56 strains of *C. difficile* isolated
- 54 strains sensitive to Moxifloxacin
- 2 strains failed to survive for testing

Toxin from direct faeces *vs.* CDIF Chrom ID™

39 culture positive strains tested		
Toxin faeces	Toxin CDIF Chrom ID™ plate	
Pos	Pos	20
Pos	Neg	2
Neg	Eq	5
Neg	Pos	8
Neg	Neg	1
Eq	Pos	2
Eq	Eq	1
Total faeces 25	Total chrom ID™ 36	39

Culture positive after 20 hours

- Gram stain
- Set up a moxifloxacin disc using 0.5 McFarland solution on Mueller Hinton Sheep agar (24 hours)
- Then increase to 3 McFarland and innoculate 300uL of suspension into Vidas Cl diff A and B strip
- If moxifloxacin <14 mm zone
  – Sent out for ribotyping
  – Notify infection control and treating doctor of potential risk

Melbourne Pathology old protocol
- If a doctor requests *C.difficile*, both culture and toxin performed
- If *C.difficile* not requested but
  – IP or NH with liquid or unformed stool –*C.diff* culture
- If bloody - toxin also done
- On all patients
- If bloody, mention of antibiotics or colitis –*C.diff* culture
- If culture positive – toxin performed

Melbourne Pathology new protocol
Liquid or unformed stools only for:
- inpatients
- nursing home patients
- antibiotic therapy
- history of *C.difficile*
- clinical notes " colitis"
- Toxin only performed where culture positive

Results
Sept to end October 2011
- 97 *Clostridium difficile* positive cultures
  Toxin Pos .....62 (one neg from faeces)
  Toxin Eq .....15 (one neg from faeces)
  Toxin Neg 20
- 2 Moxifloxacin resistant strains
  - one confirmed UK078 like ribotype
  - other pending result from MDU

CONCLUSION

Benefits of chromID™ CDIF
- Reduced time to detection
- Alcohol shock of faeces not necessary
- Culture reported at 20-24 hrs
- Unnecessary to confirm identification (latex)
- Toxin testing performed directly from chrom agar
- Moxifloxacin screen performed directly off chrom agar
- Selective and differential at 24 hours

REFERENCES

- Perry.J.D et al JCM Vol 49 Nov 2010 Evaluation of a chromogenic culture medium for isolation of C. difficile within 24 hours
- ASID C. difficile guidelines 2011
- Ferguson J.K et al Pathology Vol 43, No 5 Aug 2011 : C. difficile Laboratory testing recommendations

KEY POINTS

chromID® C. difficile offers:

➔ Reduced time to detection and does not require alcohol shock of faeces.

➔ Culture is reported at 20-24 hrs. Selective and differential results are available at 24 hours.

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Evaluation of a chromogenic culture medium for isolation of *Clostridium difficile* from stool sample

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INTRODUCTION

Culture of *Clostridium difficile* from stool specimens remains a gold standard in the diagnosis of *C. difficile* infections (CDI). By comparison with other rapid tests the delay to obtain a result is a major drawback. CHROM ID CD™ (bio-Mérieux, Lyon,France) is a transparent chromogenic medium for the detection of *C. difficile*. Colonies grow black within 24 h. We evaluated this medium against our homemade CCFA medium and the commercial CLOmedium™ (bio-Mérieux, Lyon, France).

MATERIALS AND METHODS

Stools were from adult inpatients of the St Luc-UCL University hospital (890 beds) suffering from diarrhoea. Stool samples, diluted in Cary-Blair medium (Copan) were inoculated on CCFA, CLO and CHROM ID CD using a 30µL loop. The CCFA and CLO media were read after 48h, the CHROM ID CD after 24h. Identification was confirmed by MALDI-TOF (Bruker) mass spectrometer. Cell cytotoxicity assay on MRC5 cells (CTA) and the combined GDHTOX A&B immunoassay (C. diff Quik Chek Complete TM) were performed for toxin detection. PCR-ribotyping was performed on all isolates using capillary gel electrophoresis. Toxin detection on colonies was performed using CTA and a molecular assay, illumigene® (Meridian).

RESULTS

A total of 86 stool specimens from 70 patients (36F/50M) collected in May 2011 were tested. *C. difficile* was isolated in 23 samples (27%). The strain was toxigenic in 15/23 cases.
Results are given in the Table below :

N	chrom ID CD	CCFA	CLO	TOXIGENIC ISOLATE	GDH +	TOX EIA +	CTA +
17	+	+	+	11/17	17	7	8
1	+	-	+	0/1	1		
1	-	+	+	1/1	1	1	1
3	+	-	-	2/3	1		
1	-	-	+	1/2			
63	-	-	-		1		

Culture was positive in 21/23 samples on CHROM ID CD™ (against 18 on CCFA and 20 on CLO) and all isolates were identified within 24 hours. However, in three cases, colonies did not appear black but were easily recognized by binocular examination. Two of these three isolates were toxigenic and belonged to the same ribotype. The toxigenic status of the isolates was correctly identified by illumigene® applied on colonies from 24h cultures. GDH correlated very well with positive cultures. Toxin EIA was positive in 8/15 stools with toxigenic isolates and CTA in 9/15.

CONCLUSION

The CHROM ID™ CD is a very sensitive medium for the isolation of *C. difficile* in stools in 24h.

KEY POINTS

→ chromID® C.difficile detects more colonies of *C.difficile* and more rapidly than conventional methods.

Comparison of Three Culture Media, including the new Chromogenic chromID C. difficile Agar, for the Isolation of *Clostridium difficile* from Fecal Specimens

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BACKGROUND

Culture of stool specimens is a key procedure for the diagnosis of *Clostridium difficile* (CD) infection. In order to diagnose CD infection from fecal samples we evaluated 3 culture media including 2 blood-cycloserine-cefoxitin media, Clostridium difficile agar (bioMeriéux) (CDA) and C. difficile selective medium (Oxoid) (CDSM), and the new chromogenic media chromID C. difficile agar (bioMeriéux) (CCA), with or without pre-treatment with ethanol.

METHODS

A total of 434 fresh stool specimens were processed using the 3 culture media and the tissue culture assay. Proportion of ethanol pre-treated vs untreated specimens was of 154/280. All cultures were anaerobically incubated at 35ºC and read at both 24 and 48 h of incubation. The gold standard was considered the combination of the 3 culture media at the two different reading times.

RESULTS

CD was detected in 65 specimens (15.0%).

S/Sp/PPV/NPV/Accuracy values (%) in specimens pre-treated with ethanol were as follows:

Table 1. *Results for 3 culture media with specimens pre-treated with ethanol (values expressed as %)*

Medium	Reading time	S	Sp	PPV	NPV	Accuracy
CDA	24h	29.2	99.2	87.5	94.9	88.3
	48h	66.7	97.7	84.2	94.1	92.9
CDSM (Oxoïd)	24h	58.3	97.7	82.4	88.4	91.6
	48h	79.2	90.0	59.4	95.9	88.3
CCA (chromID)	24h	75.0	98.5	90.0	95.5	94.8
	48h	95.8	82.3	50.0	99.1	84.4

S: Sensitivity Sp: Specificity PPV: Positive Predictive Value NPV: Negative Predictive Value

Table 2. *Results for 3 culture media with untreated specimens (values expressed as %)*

Medium	Reading time	S	Sp	PPV	NPV	Accuracy
CDA	24h	58.5	99.6	96.0	93.3	93.6
	48h	73.2	99.6	96.8	95.6	95.7
CDSM (Oxoïd)	24h	41.5	93.3	51.5	90.3	85.7
	48h	53.7	87.9	43.1	91.7	82.9
CCA (chromID)	24h	90.2	87.5	55.2	98.1	87.9
	48h	100	42.7	23.0	100	51.1

When characteristic colonies of CD on CCA at 24h from untreated specimens were redefined as those with a diameter equal or greater than 1mm, validity values were 85.4/98.3/89.7/97.5/96.4, respectively.

CONCLUSIONS

CCA was the most sensitive media although showed to have a relatively low specificity. Selection of characteristic colonies with a diameter equal or greater than 1 mm from untreated specimens cultured on CCA and read at 24 h was shown to be the most accurate procedure for the isolation of CD from stool specimens.

KEY POINTS

→ chromID® C.difficile is the most sensitive method at 24 hour incubation time.

Notes

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