ORIGINAL ARTICLE

Evaluation of CHROMagar™mSuperCARBA™ for the detection of carbapenemase-producing Gram-negative organisms

Julie Creighton and Hui Wang
Canterbury Health Laboratories, Christchurch

ABSTRACT

Introduction: Resistance to carbapenems, particularly due to acquired carbapenemases, in Gram-negative bacilli continues to disseminate worldwide. A recent increase in the isolation of carbapenemase producing organisms (CPO) at Canterbury Health Laboratories prompted the need to assess current screening methods. The aim of the study was to evaluate the performance of CHROMagar™mSuperCARBA™ (MSCA) against the current screening protocol of CHROMagar™ ESBL (CESB) and MacConkey agar with a 10 µg meropenem disc (MAC-Mero), for the recovery of CPO in faecal screening samples. We also compared three chromogenic media, MSCA, CESB and ChromID CARBA SMART (IDCA), against a challenge panel of multi-drug resistant organisms (MDRO), in order to determine the sensitivity and specificity of selection.

Methods: 100 screening samples were cultured onto each of MSCA, CESB, and MAC-Mero. Growth of any Gram-negative organism was investigated for the presence of resistance mechanisms. A panel of 50 isolates were inoculated onto each of MSCA, CESB and IDCA. Plates were incubated and examined for growth and typical morphology.

Results: For faecal screening, the specificities of CESB, MAC-Mero and MSCA were 73.9%, 96.6% and 88.6% respectively. No CPO were isolated during the study period. For the MDRO challenge, the respective sensitivity and specificity of each media were as follows: CESB 100%, 5.9%, MSCA 97.0%, 88.2%, and IDCA 90.9%, 88.2%.

Conclusion: The current screening method consisting of CESB in conjunction with MAC-Mero, was found to have the best overall performance. MSCA showed excellent sensitivity and specificity for the recovery of CPO, and would be considered a beneficial addition in an outbreak situation.

Keywords: carbapenemase, chromogenic media, carbapenemase producing organism, screening.

N Z J Med Lab Sci 2016; 70: 101-105

INTRODUCTION

Resistance to carbapenems, particularly due to acquired carbapenemases, in Enterobacteriaceae and non-glucose-fermenting Gram-negative bacilli continues to relentlessly disseminate worldwide, presenting an enormous challenge to the health sector (1-3). Co-carriage of other resistant mechanisms in these isolates can result in compromised treatment options, and high mortality rates in patients with severe infections (2,4). Carbapenemase-producing organisms (CPO) can reside in faecal flora, posing a risk to the patient of subsequent infections or a potential hazard for patient-to-patient transmission or environmental contamination (5). Early detection of infected or colonised patients is critical to enable effective patient management and to prevent spread of resistant organisms.

New Zealand (NZ) is a country with a low prevalence of carbapenem-resistant Enterobacteriaceae (CRE) and no predominant carbapenemase gene-type (6). With the exception of two recent hospital outbreaks, most cases in NZ have been acquired overseas (6-8). At Canterbury Health Laboratories the first CPO was isolated in 2008, with only three additional isolates over the next seven years. However, in the twelve months preceding this study, fourteen unique CPO were isolated from ten different patients, prompting the need to re-evaluate current screening methods.

There are no published guidelines or 'gold standard' for laboratory screening methods in NZ; however, it is vital that the laboratory use a method which is sensitive enough to detect a variety of CPO but also highly specific in order to reduce unnecessary workup. A range of chromogenic screening media, incorporating antimicrobials, carbohydrates and chromogenic substrates, have been developed for the selective differentiation of carbapenem-resistant Gram-negative bacilli (5).

CHROMagar™ mSuperCARBA™ (MSCA) has recently become available in NZ. MSCA is based on the SUPERCARBA media, but was further developed by CHROMagar™, in conjunction with Dr. Patrice Nordmann, for the detection and isolation of CRE, particularly OXA-48-producers, from clinical samples (9). Carbapenem-resistant Pseudomonas and Acinetobacter are also able to grow on this agar. ChromID CARBA SMART (IDCA), bioMérieux, France, is a bi-plate, consisting of OXA-48-like screening media (chromID OXA-48) on one side and CRE screening media (chromID CARBA) on the other. Both MSCA and IDCA can detect a variety of CPO, making them suitable for use in NZ.

The first part of the study was to evaluate the performance of MSCA against the current screening protocol of CHROMagar™ ESBL (CESB) and MacConkey agar with a 10 µg meropenem disc (MAC-Mero), for the detection of CPO in routine faecal screening samples. The second part of the study was to compare three chromogenic media, MSCA, IDCA and CESB, against a challenge set of multi-drug resistant organisms (MDRO), at low bacterial inoculum, in order to determine the sensitivity and specificity of selection.

METHODS AND MATERIALS

Routine CPO screening

Between April and May 2016, one hundred consecutive screening samples, collected from hospitalised patients, consisting of either faeces or rectal swabs, were cultured onto each of MSCA, CESB and MacConkey Agar with a 10 µg meropenem disc placed in the first quartile. All media was supplied by Fort Richard Laboratories Ltd, NZ. After overnight incubation all colony types growing on MSCA or CESB, or within 25 mm of the meropenem zone, were investigated further. Isolate identification was performed using Bruker MALDI-TOF mass spectrometry (Bruker Daltonics Inc, Billerica, USA). ESBL production was tested for using a double disk synergy test and AmpC production tested for using a combination disk test with cloxacillin, as previously described (10). Pseudomonas or Acinetobacter were tested for susceptibility to meropenem by disc diffusion, whereas Enterobacteriaceae had susceptibility testing performed with the Phoenix™ automated turbidometric growth detection system (BD Diagnostics, Sparks, USA). Interpretation criteria for susceptibility tests were applied according to EUCAST 2016 (The European Committee on Antimicrobial Susceptibility Testing) guidelines and incorporating Phoenix BDXpert rules. Any carbapenem-resistant isolate was investigated for phenotypic carbapenemase production using an in-house CarbaNP test and the carbapenem inhibition method (CIM), as previously described (10,11).

Challenge MDRO

The challenge bacterial panel consisted of fifty non-duplicate multi drug-resistant strains, including 36 Enterobacteriaceae, seven *Pseudomonas aeruginosa* and seven *Acinetobacter baumannii*. Among these isolates there were 33 carbapenemase-producing strains: NDM (*n* = 11), KPC (*n* = 4), VIM (*n* = 3), IMP (*n* = 3), OXA-48-like (*n* = 5, including one strain that coproduced NDM-1), OXA-23 (*n* = 3) and one each of OXA-24, OXA-25, OXA-27, OXA-58; and 17 non-carbapenemase producing isolates that produced other resistant mechanisms such as extended spectrum β-lactamase (ESBL), plasmid-mediated AmpC, AmpC-hyper-production or K1 chromosomal β-lactamase. Six of the 17 non-carbapenemase strains were non-susceptible to one or more carbapenem. All bacterial isolates used in the study were either characterised clinical isolates obtained from Canterbury Health Laboratories or reference strains provided by the Institute of Environmental Science and Research Limited (ESR). *Klebsiella pneumoniae* BAA1705 (KPC positive) and *K. pneumoniae* BAA1706 (KPC negative/ESBL positive) were included in the panel and used as positive and negative controls respectively. Isolates were stored at -80°C and subcultured twice onto blood agar before tests were performed.

To obtain a low-bacterial load, each isolate was suspended in sterile saline to 0.5 McFarland standard, then 20 µl was further diluted into 2 ml of saline. A 10 µl loopful of this suspension was inoculated onto each of MSCA, IDCA and CESB. Plates were incubated at 36°C and examined for growth and typical morphology after 24 hours incubation and again at 48 hours.

RESULTS

Routine CPO screening

In total, 100 screening samples, from 88 patients, were included in the trial comparing the current protocol of CESB and MAC-Mero with MSCA. No CPO were found during the study period, so sensitivity limits were not able to be determined for this part of the study. In total, nine ESBL-producing Enterobacteriaceae were recovered from CESB agar, resulting in a prevalence of 10.2%. CESB grew twenty five non-CPO strains, from 23 patients, resulting in a specificity of 73.9% for the detection of CPO (Table 1).

Table 1. Gram-negative organisms recovered on screening media from clinical samples.

Organism	Resistance mechanism	CESB[1]	MAC-Mero[2]	MSCA[3]	MER[4]	ERT[5]	CarbaNP and CIM
E. coli	ESBL	8	0	0	S	S	
K. pneumoniae	ESBL	1	0	0	S	S	
E. aerogenes	AmpC	1	1	1	R	R	-
E. aerogenes	AmpC	1	0	0	S	S	
E. cloacae	AmpC	1	0	1	S	R	-
E. coli	AmpC	1	0	1	S	S	
Citrobacter spp	AmpC	3	0	0	S	S	
P. aeruginosa	Porin/efflux	5	0	5	S	S	
A. baumannii	Intrinsic	2	0	2	S	NA	
S. maltophilia	Intrinsic	2	2	2	NA	NA	
Total		25	3	12			
Specificity		73.9%	96.6%	88.6%			

[1] CESB = CHROMagar™ ESBL; [2] MAC-Mero = MacConkey agar with 10µg meropenem disc; [3] MSCA = CHROMagar™ mSUPER CARBA; [4] MER = meropenem; [5] ERT = ertapenem;
[6] The number of patients whose samples were positive for the resistant organisms were: CESB 23, MAC-MERO 3 and MSCA 10.

Amongst the 25 strains, eight isolates were ESBL-producing *Escherichia coli*, one isolate was an ESBL-producing *K. pneumoniae* and seven isolates were AmpC-hyperproducing Enterobacteriaceae, including three *Enterobacter* species which were resistant to one or more carbapenem, but tested negative for carbapenemase production. In addition, five *P. aeruginosa* and two *A. baumannii* also grew on CESB; all of which were susceptible to meropenem and ESBL negative. Two isolates identified as *Stenotrophomonas maltophilia* were not tested beyond an identification. All of these strains contained resistant mechanisms that would account for growth on CESB medium.

Only three non-CPO were found to be growing within a 25 mm zone on the MAC-Mero screen (96.6% specificity), including a carbapenem-resistant *Enterobacter aerogenes*, and two *S. maltophilia* (Table 1).

MSCA was a more effective inhibitor of non-CPO than CESB, with a specificity of 88.6%. Twelve strains, from 10 patients were isolated, including three AmpC-hyperproducing Enterobacteriaceae, five *P. aeruginosa* and two *A. baumannii* and two *S. maltophilia* (Table 1). None of the nine ESBL-producing Enterobacteriaceae was recovered on MSCA.

Table 2. Performance of CHROMagar ESBL(CESB), CHROMagar mSuperCARBA (MSCA) and chromID CARBASMART (IDCA) for the detection of multi-drug resistant organisms.

			Chromogenic Screening media			
					ChromID CARBA	
Resistance mechanism(s)	**Species**	**n**	**CESB**	**MSCA**	**CARBA**	**OXA-48**
Carbapenemase-producing organisms						
KPC	*K. pneumoniae*	4	+[1]	+	+	-[2]
NDM-1	*K. pneumoniae*	1	+	+	+	-
NDM-1	*P. stuartii*	1	+	+	+[3]	-
NDM-1, ESBL	*P. mirabilis*	1	+	+[3]	-	-
NDM-1, ESBL	*E. coli*	1	+	+	+	-
NDM-1	*E. coli*	2	+	+	+	-
NDM-1	*P. aeruginosa*	1	+	+	+	-
NDM-1	*K. oxytoca*	1	+	+	+	-
NDM-5	*K. pneumoniae*	1	+	+	+	-
NDM-5	*K. oxytoca*	1	+	+	+	-
NDM-5	*E.coli*	1	+	+	+	-
IMP-4	*E. coli*	1	+	-	-	-
IMP-4	*K. pneumoniae*	1	+	+	+	-
IMP-7	*P. aeruginosa*	1	+	+	+	-
VIM-2	*P. aeruginosa*	1	+	+	+	-
VIM-4	*P. aeruginosa*	1	+	+	+	-
VIM-5	*P. aeruginosa*	1	+	+	+	-
OXA-48	*K. pneumoniae*	2	+	+	+	+
OXA-48/NDM-1	*K. pneumoniae*	1	+	+	+	+
OXA-181	*K. pneumoniae*	1	+	+	+	+
OXA-232	*K. pneumoniae*	1	+	+	+	+
OXA-23	*A.baumannii*	1	+	+	-	-
OXA-23	*A.baumannii*	2	+	+	+	+
OXA-24	*A.baumannii*	1	+	+	+	-
OXA-25	*A.baumannii*	1	+	+	+	+
OXA-27	*A.baumannii*	1	+	+	-	+
OXA-58	*A.baumannii*	1	+	+	-	+
Non carbapenemase-producing organisms						
ESBL	*K. pneumoniae*	2	+	-	-	-
ESBL	*E. coli*	4	+	-	-	-
ESBL	*P. mirabilis*	1	+	-	-	-
ESBL	*P. aeruginosa*	1	+	+	+	+
ESBL/AmpC-hyperproducer	*E. coli*	1	+	-	-	-
ESBL/AmpC-hyperproducer	*C. freundii*	1	+	-	-	-
ESBL/AmpC-hyperproducer	*E. cloacae*	1	+	-	-	-
K1	*K. oxytoca*	1	+	-	-	-
ACC-type plasmid AmpC	*P. mirabilis*	1	+[3]	-	-	-
plasmid AmpC	*P. mirabilis*	1	-	-	-	-
AmpC-hyperproducer	*E. cloacae*	1	+	-	-	-
AmpC-hyperproducer	*E. cloacae*	1	+	+	-	-
Porin/efflux	*P. aeruginosa*	1	+	-	+	-
Total		**50**				
Sensitivity for CPO			100%	97.0%	90.9%	
Specificity for CPO			5.9%	88.2%	88.2%	

n = number of strains tested.
[1] + Growth and colour morphology as expected after 24 hours.
[2] – No growth after 48 hours.
[3] + Growth only after 48 hours.

Challenge MDRO

CESB, MSCA and IDCA were compared for sensitivity of detection for 33 CPO and inhibition of 17 non-CPO culture strains (Table 2). CESB was the most sensitive, detecting 33/33 (100%) of the carbapenemase-producing strains. In addition, 11/11 ESBL-producing strains were also detected on CESB. One *Proteus mirabilis* harbouring a plasmid-mediated AmpC failed to grow on CESB after 48 hours and an ACC-producing *P. mirabilis* grew only scanty colonies after 48 hours. All of the study strains would be expected to grow on CESB; however for the purposes of detecting CPO, this media would have a specificity of just 5.9%.

MSCA showed excellent sensitivity, with 31/33 (93.9%) of the carbapenemase-producing organisms growing after 24 hours incubation and all strains displaying colonial morphology as indicated by the manufacturer. A NDM-1-producing *Proteus mirabilis* grew just one colony after 48 hours, bringing the final sensitivity to 97%. An IMP-4-producing *E.coli* failed to grow after 48 hours and repeat testing produced the same result. Of the seventeen non-CPO, one ESBL-producing *P. aeruginosa* and one AmpC- hyperproducing *Enterobacter cloacae* both grew on MSCA (88.2% specificity). Both of these isolates were highly resistant to meropenem (MIC of >32 mg/L and 16 mg/L respectively) but were phenotypically and genetically negative for carbapenemase.

The IDCA bi-plate had the lowest sensitivity for CPO detection, at 90.9%. On the OXA-screen section, all five of the OXA-48-like isolates grew but two of the oxacillinase-producing *A. baumannii* strains failed to grow. The OXA-screen was very specific for oxacillinase producers, inhibiting all of the remaining study strains, except for one ESBL-producing *P. aeruginosa*. On the CARBA section, 27/33 CPO grew after 24 hours (81.8% sensitivity). A NDM-1-producing *Providencia stuartii* grew a few colonies after 48 hours, giving a final sensitivity of 84.8%. Five CPO isolates failed to grow after 48 hours, with repeat testing producing the same results. Of note is that the growth of several study strains tended to be less plentiful on IDCA than growth on MSCA. Among the non-CPO group, only two carbapenem-resistant *P. aeruginosa* grew on the CARBA side, giving a specificity of 88.2%.

DISCUSSION

Rapid and accurate identification of patients colonised with CPO is critical to control the spread of nosocomial infections and to initiate correct antimicrobial therapy. Although current reports of CPO in NZ are rare, most cases have links to overseas hospital care or travel (12,13). Active surveillance of these high-risk patients as well as critical care areas such as ICU and Bone Marrow Transplant Units is prudent in order to prevent CPO becoming endemic in NZ.

There is no 'gold standard' method for surveillance testing. Current molecular methods, used directly on clinical samples, offer sensitive and rapid results, but this technology is expensive, is limited to the detection of known genes and organisms are not available for identification or antimicrobial profiles. Chromogenic screening agars are useful culture-based methods, but have primarily been developed for the recovery of CRE or even for a geographical area with a predominating gene -type e.g. KPC producers in the USA (5), rather than for a diverse range of CPO. The Centers for Disease Control and Prevention has recommended the use of an overnight broth enrichment step for the recovery of CRE, but the slight increase in sensitivity may not be sufficient to outweigh the disadvantage of prolonged time-to-detection or a resulting decrease in specificity (14,15).

At Canterbury Health Laboratories, we consider that the detection of carbapenemase-producing Pseudomonas and Acinetobacter is also important (3). *A. baumannii* strains that harbour oxacillinases may not be a significant infection control concern, as oxacillinases are rarely found in other genera, but there is still potential for nosocomial spread and infection. *P. aeruginosa* is an important nosocomial pathogen, especially in vulnerable patients, and strains can harbour mobile genetic elements (3). CESB is an essential part of our routine screening so that patients who are colonised with ESBL producers are identified. However, most ESBL-type screening media contain cefpodoxime, potentially inhibiting those CPO with low carbapenem MICs and no coproduction of ESBL (9). In addition, media designed for ESBL detection maybe inefficient for CPO recovery in areas that have high prevalence rates of ESBL or AmpC-hyperproducing Enterobacteriaceae (15).

The finding of a 10.2% prevalence rate for ESBL producers in the patient screening study is higher than we would have predicated, based on annual prevalence rates of <4% in clinical isolates. However, our study is small and this rate of ESBL prevalence may be over-estimated. Even so, this rate of ESBL prevalence should not hinder the use of an ESBL-based screening media for the detection of CPO in our current setting.

Our study found that using CESB in conjunction with MAC-Mero would be sensitive for the detection of ESBL producers from patient samples, and would also recognise potential CPO within the meropenem zone. Furthermore, the inclusion of a non-selective agar such as MacConkey has the added benefit of acting as an 'honesty' plate, whereby the growth of faecal flora can confirm adequate sample collection. MSCA also performed well in this part of the study, with the resulting specificity of 88.6% being higher than that found in earlier studies using the original SUPERCARBA formulation (9,16). Of note is that only 3/12 of the non-CPO recovered were Enterobacteriaceae, which would have resulted in a much higher specificity had only detection of CRE been evaluated.

The second part of our study, evaluating MSCA, IDCA and CESB against fifty MDRO, found that CESB had superior sensitivity (100%) for the detection of CPO. However, the ability of non-CPO to grow so well on this media may mask the growth of low numbers of CRE in a clinical setting, or fail to grow CRE that are susceptible to cefpodoxime. In comparison, the final sensitivity of MSCA was 97%, with a specificity of 88.2%, which compares well with earlier SUPERCARBA evaluations, using a diverse range of CRE (9,16,17). IDCA was the least sensitive agar for the recovery of CPO, with a combined sensitivity of 90.9%. This result is similar to that found by Girlich et al when they compared chromID CARBA plus chromID OXA-48 with SUPERCARBA for the detection of OXA-48-like producing Enterobacteriaceae (18).

A Belgium study by Heinrichs *et al.* evaluated chromID CARBA and Brilliance CRE (Oxoid, ThermoFisher), comparing a direct plating method and a MacConkey broth pre-enrichment step, on 730 rectal swabs (19). Their study found a slightly improved sensitivity for the detection of OXA-48-like producing Enterobacteriaceae with the enrichment step, but the 24 hour time-to-detection delay was a disadvantage. In contrast to our finding, they reported that incubation beyond 24 hours did not increase recovery of CRE, but had the added disadvantage of decreased specificity.

Screening of patients for ESBL and CPO colonisation should be part of a package of intervention strategies used to limit the spread of MDRO and appropriate choice of chromogenic media will ultimately depend on local epidemiology, including established MDRO types.

Limitations of this study include the relatively small number of screening samples tested and the lack of any carbapenemase-producing isolates recovered during the study period. We did not test multiple combinations of CPO on a single plate, which could well mimic clinical samples, when comparing the sensitivity and specificity of the three screening media. This may have affected overall performance.

In summary, our study has shown that CESB used in conjunction with MAC-Mero performs with high combined sensitivity and specificity for the detection of ESBL and CPO in the New Zealand setting. MSCA also performed with excellent sensitivity for the detection of CPO and would be a useful addition to the screening strategy during a CPO outbreak or if local MDRO prevalence rates were to significantly increase.

ACKNOWLEDGMENTS

We are grateful to FRL for supplying the CHROMagar mSuperCARBA used in this study.

AUTHOR INFORMATION

Julie Creighton, DipMLT, Senior Medical Laboratory Scientist[1] and Clinical Lecturer[2]
Hui Wang, PGDipMLSc, Senior Medical Laboratory Scientist[1]

[1]Department of Microbiology, Canterbury Health Laboratories, Christchurch
[2]University of Otago, Christchurch

Author for correspondence: Julie Creighton, Microbiology Laboratory, Canterbury Health Laboratories Limited, P. O. Box 151, Christchurch, New Zealand.
Email: julie.creighton@cdhb.health.nz

REFERENCES

1. Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. *Clin Microbiol Infect* 2014; 20: 821-830.
2. Tängdén T, Giske CG. Global dissemination of extensively drug-resistant carbapenemase-producing Enterobacteriaceae: clinical perspectives on detection, treatment and infection control. *J Intern Med* 2015; 277: 501 -512.
3. Gniadek TJ, Carroll KC, Simner PJ. Carbapenem-resistant non-glucose-fermenting Gram-negative bacilli: the missing link to the puzzle. . *J Clin Microbiol* 2016; 54: 1700-1710.
4. Tzouvelekis LS, Markogiannakis A, Psichogiou M, Tassios PT, Daikos GL. Carbapenemases in Klebsiella pneumoniae and other Enterobacteriaceae: an evolving crisis of global dimensions. *Clin Microbiol Rev* 2012; 25: 682-707.
5. Viau R, Frank KM, Jacobs MR, Wilson B, Kaye K, Donskey CJ, et al. Intestinal carriage of carbapenemase-producing organisms: current status of surveillance methods. *Clin Microbiol Rev* 2016; 29: 1-27.
6. Institute of Environmental Science and Research Ltd. Enterobacteriaceae with acquired carbapenemases, 2015. Porirua, New Zealand: ESR; 2016. Available at https://surv.esr.cri.nz/PDF_surveillance/Antimicrobial/ACE/2015Carbap.pdf
7. Munroe S. First recognised case of patient to patient transmission of carbapenemase-producing Enterobacteriaceae in a New Zealand health care setting. ASA 2016, poster P02.1.
8. Howard J, Creighton J, Heffernan H, Williamson D, Schousboe M, Metcalf S, et al. The first outbreak of a New Delhi Metallo-ß-Lactamase (NDM) producing organism in a New Zealand healthcare facility. ECCMID 2016, poster P0689.
9. Nordmann P, Girlich D, Poirel L. Detection of carbapenemase producers in Enterobacteriaceae by use of a novel screening medium. *J Clin Microbiol* 2012; 50: 2761-2766.
10. Creighton J, Jayawardena S. Comparison of four phenotypic tests, three biochemical tests and Cepheid Xpert Carba-R for detection of carbapenemase enzymes in Gram-negative bacteria. *NZ J Med Lab Sci* 2015; 69: 81-88.
11. van der Zwaluw K, de Haan A, Pluister GN, Bootsma HJ, de Neeling AJ, Schouls LM. The carbapenem inactivation method (CIM), a simple and low-cost alternative for the Carba NP test to assess phenotypic carbapenemase activity in Gram-negative rods. *PLoS One* 2015; 10: e0123690.
12. Heffernan H, Dyet K, Munroe S, Creighton J, Chan S, Taylor S, et al. First cases of KPC-type carbapenemase-producing bacteria in patients in New Zealand hospitals. *J Global Antimicrob Resist* 2014; 2: 330-333.
13. Creighton J, Heffernan H, Howard J. Isolation of seven distinct carbapenemase-producing Gram-negative organisms from a single patient. *J Antimicrob Chemother* 2016; (in press): doi: 10.1093/jac/dkw378.
14. CDC (2009). Laboratory protocol for detection of carbapenem-resistant or carbapenemase-producing, *Klebsiella* spp and *E.coli* from rectal swabs. Atlanta, GA: Centers for Disease Control and Prevention. http://www.cdc.gov/HAI/pdfs/labSettings/Klebsiella_or_Ecoli.pdf
15. Wilkinson KM, Winstanley TG, Lanyon C, Cummings SP, Raza MW, Perry JD. Comparison of four chromogenic culture media for carbapenemase-producing Enterobacteriaceae. *J Clin Microbiol* 2012; 50: 3102-3104.
16. Girlich D, Bouihat N, Poirel L, Benouda A, Nordmann P. High rate of faecal carriage of extended-spectrum β-lactamase and OXA-48 carbapenemase-producing Enterobacteriaceae at a University hospital in Morocco. *Clin Microbiol Infect* 2014; 20: 350-354.
17. Girlich D, Poirel L, Nordmann P. Comparison of the SUPERCARBA, CHROMagar KPC, and Brilliance CRE screening media for the detection of Enterobacteriaceae with reduced susceptibility to carbapenems. *Diagn Microbiol Infect Dis* 2013; 75: 214-217.
18. Girlich D, Anglade C, Zambardi G, Nordmann P. Comparative evaluation of a novel chromogenic medium (chromID OXA-48) for detection of OXA-48 producing Enterobacteriaceae. *Diag Microbiol Infect Dis* 2013; 77: 296-300.
19. Heinrichs A, Nonhoff C, Roisin S, De Mendonca R, Adam AS, et al. Comparison of two chromogenic media and enrichment broth for the detection of carbapenemase-producing Enterobacteriaceae on screening rectal swabs from hospitalized patients. *J Med Microbiol* 2016; 65: 438-441.