P-112

Evaluation of Four Media for the Isolation of Resistant Gram Negative Organisms from Surveillance Rectal Swabs

Natasha Joshi-Caesar, Gloria Ricci, David Richardson

William Osler Health System, Brampton, Canada

Email: natasha.joshi@williamoslerhs.ca

WILLIAM OSLER HEALTH SYSTEM

Brampton Civic Hospital
Microbiology Department
2100 Bovaird Dr E
Brampton, Ontario, L6R 3J7
(905) 494-2120, Ext 58680

Abstract (abbreviated)

Background: William Osler Health System serves a diverse population with a very high rate of immigration from South Asia. We see high rates of Gram negative resistance in patients admitted to our hospitals. **Objective:** To evaluate ESBL Isolation™ agar with 2mg cefpodoxime/ml (OXOID), Brilliance™ agar (OXOID), CHROMagar™ C3GR agar (Alere) and ChromID™ ESBL agar (bioMerieux) media for detection of multi-resistant gram-negative organisms from rectal surveillance swabs.

Methods: 161 rectal swabs from patients in critical care units or patients from non-critical care areas with travel within 3 months were tested. The rectal swabs were placed in 2.0 ml sterile saline and vortexed to obtain a broth suspension. 50µl of the broth was inoculated on each of the 4 agars. The agars were incubated as per manufacturer's recommendations. Distinct colony types from each media were sub-cultured onto Columbia Blood agar (BA) and MacConkey agar (MAC). Growth from the sub-cultured BA and MAC was used for identification and antimicrobial susceptibility testing (AST) on bioMerieux Vitek® 2. Isolates positive for Extended Spectrum Beta Lactamases were further tested using the combination disc method.

Conclusions:
OXOID ESBL Isolation™ agar isolated the most ESBL and AmpC organisms. CHROMagar™ C3GR performed similarly to ESBL Isolation agar for ESBLs and also isolated the widest variety of *Enterobacteriaceae* with increased resistance. This may be an important consideration from a surveillance perspective. The one NDM1 *Klebsiella pneumoniae* organism isolated during the study grew on all media.

Introduction

William Osler Health System (WOHS) consists of two large community hospitals that serve a diverse population with a high rate of immigration from South Asia. With our immigration patterns and our proximity to a large international airport we care for many patients with recent travel to South Asia. Rates of gram negative resistance in our hospitals has been increasing and currently 18% of *E. coli* bacteremias are extended-spectrum beta-lactamase producing (ESBL) *E. coli*. The prevalence of ESBL rectal carriage in returning travelers to our hospital corporation is greater than 75%, which is similar to rates of carriage in India (1). Carbapenem-resistant *Enterobacteriaceae* (CRE) are now endemic in South Asia (2) and becoming increasingly identified in patients returning from abroad to WOHS. In Ontario, Canada, our Provincial Infectious Diseases Advisory Committee (PIDAC) recommends that all health care facilities institute a screening program and targeted surveillance for CRE (3).

Objective

To evaluate four commercially available primary culture media for the detection of multi-resistant gram negative organisms, including CREs from rectal surveillance swabs. The four media considered for evaluation were ESBL Isolation™ agar (OXOID), OXOID Brilliance™ agar, CHROMagar™ C3GR agar (Alere) and ChromID™ ESBL agar (bioMerieux). All except the OXOID ESBL Isolation™ agar are chromogenic.

Materials and Methods

<u>Method 1:</u> 161 rectal surveillance swabs from patients with a travel history to India or Pakistan or admissions in critical care areas of CCU, ICU, Neonatal Care Unit and Special Care Nursery from either Brampton Civic or Etobicoke General sites, were considered for the study. Patients with an admission to a hospital outside of Canada in the last 12 months were also selectively included in this evaluation.

Each rectal swab was placed in 0.85% sterile saline and vortexed to obtain a broth suspension. 50 µl of the broth suspension was pipetted onto each of the four agars and streaked for isolation using a sterile loop or an automatic streaker such as Isoplater® (Vista Technology Inc). The agars were incubated in ambient air, protected from light, at 35°C as per manufacturer's recommendations. Plates were examined and results recorded after the recommended incubation periods (24-48 hours). Distinct, isolated colonies were sub-cultured onto Sheep Blood Agar, SBA (OXOID) and MacConkey agar, MAC (OXOID) and incubated at 35°C in ambient air for 18-24hours. Growth from the sub-cultured SBA was used for further testing. For each isolate, spot oxidase (BD, BBL™) and indole (BD, BBL™) tests were performed and further Identification and antimicrobial susceptibility testing (AST) were done using the bioMerieux Vitek® 2 System. Isolates flagged by the Vitek® 2 System as positive for extended spectrum β-lactamases (ESBL) were further tested using the CLSI combination disc method specified in M100-S20 M2 (4), to establish ESBL or AmpC β-lactamase resistance patterns. Organisms flagged with carbapenem resistance were referred to the Public Health Laboratory in Toronto for further characterization by molecular methods.

<u>Method 2:</u> 100 gram negative isolates of previously established resistance patterns from various body sites (urine, rectal, genital, toe, thigh, leg, abdominal aspirate) were cultured retrospectively on SBA after being stored in glycerol at -70C. Using fresh, isolated colonies, a homogenous broth suspension was prepared in 0.85% sterile saline, equivalent to 0.5 McFarland. A 1:10 dilution was prepared from the standardized broth suspension and vortexed to mix. Each agar was inoculated with 10µl of the diluted suspension and streaked for isolation using a sterile loop. Following manufacturer

Results

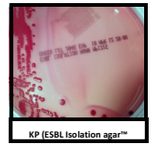
Table 1: Evaluation of Chromogenic/Colonial Appearance

Organism	ESBL Isolation™ Non-chromogenic	OXOID Brilliance™ Chromogenic	CHROMagar™C3GR Chromogenic	ChromID™ ESBL Chromogenic
Escherichia coli	Pink (As expected)	Dark Blue (with Green halo)	Mauve (As expected)	Mauve or Buff brown
Klebsiella pneumoniae		Green (As expected)	Metallic Blue Or Metallic Blue (with Pink halo)	Green (As expected)
Morganella spp	Inhibited	Inhibited	Translucent	Inhibited
Enterobacter spp	Pink (As expected)	Green (As expected)	Metallic Blue	Mauve (Expected to be Green)
Citrobacter spp			Metallic Blue	Inhibited (expected to be Green)
Pseudomonas spp	Colourless or Green (As expected)	Colourless	Colourless, clear	Buff or brown (As expected)
Acinetobacter spp	Colourless	Colourless	Colourless	Buff (As expected)
Proteus spp	Colourless	Tan (Brown halo not observed)	Tan (brown halo)	Inhibited (expected to grow brown)

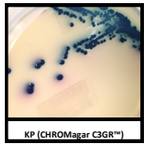
Table 3: Sensitivity Results (based on organisms of known resistance)

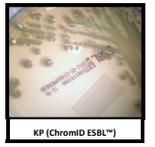
Organism	Total Number of Organisms in Study	ESBL Isolation ™	OXOID Brilliance ™	CHROMagar ™ C3GR	ChromID™ ESBL
NDM1 KP**	13	100%	100%	100%	100%
NDM1 EC**	5	100%	100%	100%	100%
NDM1 *P. stuartii*	1	100%	Inhibited	100%	Inhibited
OXA-48 KP**	2	100%	100%	100%	100%
NDM1 *M. morganii*	1	100%	100%	100%	100%
MDR*** *P.putida*	1	100%	100%	100%	100%
ESBL EC**	51	98%	92%	100%	98%
AmpC EC**	21	100%	14%	100%	29%*
ESBL/AmpC EC**	21	100%	100%	100%	100%
ESBL KP**	3	100%	100%	100%	100%
Overall Sensitivity		99%	77%	100%	82%

One AmpC EC grew only after 48 hours incubation, ** *Escherichia coli* EC, *Klebsiella pneumonia* KP
*** Multidrug resistant MDR

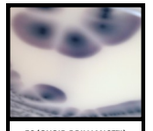

KP (ESBL Isolation agar™

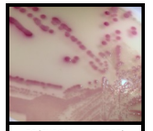

KP (OXOID BRILLIANCE™)


KP (CHROMagar C3GR™)


KP (ChromID ESBL™)


EC (ESBL Isolation agar™


EC (OXOID BRILLIANCE™)


EC (CHROMagar™ C3GR)


KP (ChromID ESBL™)

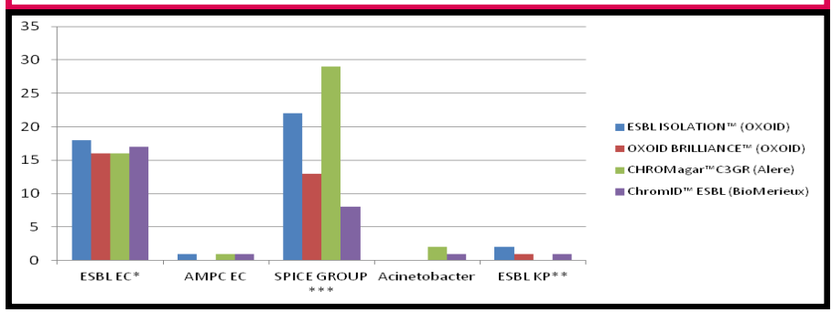
Results

Table 2: Summary of Results from 96 Rectal Swabs

Organism	Total Numbers Isolated From All Media)	ESBL Isolation™ Non-chromogenic		OXOID Brilliance™ Chromogenic		CHROMagar™C3GR Chromogenic		ChromID™ ESBL Chromogenic	
ESBL EC*	18	(17/18)	94%	(16/18)	89%	(16/18)	89%	(17/18)	94%
ESBL KP**	1	(1/1)	100%	(1/1)	100%	0		(1/1)	100%
AmpC EC*	2	(2/2)	100%	0		(1/2)	50%	(1/2)	50%
***Morganella* spp**	1	0		0		(1/1)	100 %	0	
***Enterobacter* spp**	16	(8/16)	50%	(5/16)	31%	(15/16)	93%	(1/16)	6%
***Citrobacter* spp**	6	(4/6)	67%	0		(5/6)	83%	0	
***Pseudomonas* spp**	16	(10/16)	62%	(8/16)	50%	(8/16)	50%	(7/16)	44%
***Acinetobacter* spp**	2	0		0		(2/2)	100 %	(1/2)	50%

***Escherichia coli* EC, ****Klebsiella pneumoniae* KP**

Figure 1: Number of Organism Types Isolated by Each Media from Rectal Swabs



***Escherichia coli* EC, ****Klebsiella pneumoniae* KP, ***SPICE GROUP: *Serratia* spp, *Pseudomonas aeruginosa*, Indole positive *Proteae* (*Proteus vulgaris*, *Morganella morganii*, *Providencia* spp), *Citrobacter* spp, *Enterobacter* spp.**

Discussion

WOHS performs surveillance to identify carriers of ESBL, and CRE organisms from rectal swabs. We conducted a comparative study of four media suitable for primary culture. ESBL Isolation™ was the only non-chromogenic agar that was evaluated in our study. All media contained cefpodoxime. Additionally, the three chromogenic media also had a proprietary antibiotic mixture and differentiation between organisms was achieved by the inclusion of chromogens. To accommodate bench and media supply constraints, a few of the 161 swabs included in our study could not be tested on all four media. Therefore, the final evaluation was based on the remainder of the swabs that were tested on all four media (96). The total numbers of organisms isolated from all media, throughout the study were used as a baseline to compare the performance of each media (Table 2). ESBL Isolation™ agar and ChromID™ ESBL isolated the highest number of ESBL organisms. CHROMagar™ C3GR failed to grow the only ESBL KP isolated in our study however, performed just as well as OXOID Brilliance™ in isolating ESBL EC. CHROMagar™ C3GR isolated the most organisms from the SPICE group, *Enterobacter* spp, *Citrobacter* spp and *Acinetobacter* spp whereas ChromID™ ESBL was most inhibitory for these organisms (Figure 1, Table 2). *Acinetobacter* was inhibited on the ESBL Isolation™ agar and OXOID Brilliance™ agar. In addition to the organisms noted above, we also isolated NDM1-KP, *Providencia* spp, *Hafnia* spp, and *Proteus* spp during our study. There was minimal breakthrough of *Enterococcus* and yeast and other gram positive organisms were inhibited.

One-hundred previously characterized resistant gram negative organisms were tested in a second study (Method 2) to compare the sensitivity of the media for organisms considered relevant under our surveillance criteria (Table 3). Overall, CHROMagar™ C3GR had the highest sensitivity and was followed closely by ESBL Isolation™ agar. EC with plasmid-mediated AmpC were isolated with 100% sensitivity on ESBL Isolation™ agar and CHROMagar™ C3GR agar, whereas OXOID Brilliance™ and ChromID™ both had low sensitivity for these organisms contributiong to their overall lower sensitivity.

With regards to differential properties ("Ease of Use"), ESBL Isolation™ agar facilitated the differentiation between lactose fermenters (LF) and non-lactose fermenters (NLF), and mucoid versus non-mucoid colonies. However, due to limited differential properties of the media the ability to differentiate between LF with similar appearance was compromised. Overall, same organisms consistently appeared with similar colour and size properties on CHROMagar C3GR™ agars and it was easy to differentiate between organism types. On ChromID™ ESBL, EC appeared to be either mauve or tan. Some colonies of EC that appeared tan after 24 hours appeared mauve at 48 hours (Table 1). Likewise colonies of EC appeared either blue or pink on OXOID Brilliance™. EC was considered an organism of interest and for this organism the colour was not always reliable as a differentiating factor for either of those two media. On ChromID™ ESBL *Enterobacter* spp did not grow as expected (green) and appeared similar to EC (mauve) in our study (Table 1). Additionally, on these two media *Proteus* spp and *Morganella* spp, did not utilize chromogens and appeared tan or brown on the media and were difficult to differentiate based on their colonial appearance.

Conclusion

- **All four media tested had excellent performance for the isolation of ESBL organisms from surveillance rectal swabs.**
- **ChromID™ ESBL (bioMerieux) was the most inhibitory media for the SPICE group of organisms.**
- **CHROMagar™ C3GR was the least inhibitory media for organisms from the SPICE group.**
- **The four media tested all had excellent performance for the isolation of known CRE organisms.**
- **The OXOID Brillance™ (OXOID) and ChromID™ ESBL (bioMerieux) media had low sensitivity for the detection of plasmid-mediated AmpC EC.**

The selection of suitable media depends on the surveillance criteria of the institution. As per the manufacturers technical data sheets (6)(7)(8)(9), all four media are purpose made for isolation of ESBL organisms. To meet surveillance needs of our unique patient population we required media that isolated the most ESBL and plasmid-mediated AmpC organisms. In addition, it was important that we were able to detect CRE organisms, which can occur in many members of *Enterobacteriacae*. Therefore, we required media that was able to isolate organisms from the SPICE group as well. The turnaround time for a negative result was also considered in our selection criteria. The OXOID Brillance™ (OXOID) and ChromID™ ESBL media (bioMerieux), both required 48 hours of incubation to obtain a negative result whereas ESBL Isolation ™ (OXOID) and CHROMagar™ C3GR (Alere) required 24 hours. CHROMagar™ C3GR (Alere) also had the best differential properties compared to the other three media evaluated. Based on our studies from direct rectal swabs and organisms of known resistance CHROMagar™ C3GR (Alere) met most of our surveillance requirements.

References

1. Tangden T, Cars O, Melhus A, Lowdin E. Antimicrob Agents Chemother. 2010 Sep;54(9):3564-8.
2. **Nordmann P, Poirel L, Walsh TR, Livermore DM. Trends Microbiol. 2011 Dec;19(12):588-95.**
3. **Public Health Ontario: Provincial Infectious Diseases Advisory Committee. 2012 Feb: http://www.oahpp.ca/resources/documents/pidac/Annex%20A%20-%20PHO%20template%20-%20REVISION%20-%202012Apr25.pdf.**
4. **CLSI. Performance Standards for Antimicrobial Susceptibility Testing: 2010;CLSI document M100-S20:M2.**
5. **NCCLS. Quality Control for Commercially Prepared Microbiological Culture Media: Approved Standard-Third Edition. 2004 June: NCCLS document M22-A3.**
6. **CHROMagar™ C3GR supplement, Notice 091014.**
7. **OXOID Brilliance ESBL™ Technical Data Sheet.**
8. **bioMerieux ChromID™ ESBL agar. REF43481; 13594 B - en - 2006/10.**
9. **OXOID ESBL Isolation Agar (MP1315)Technical Data Sheet.**