C-252

Evaluation of CHROMagar KPC™ and Other Selective Media for Surveillance of Carbapenemase-producing *Enterobacteriaceae* and Multi-drug Resistant *Acinetobacter* species

A. Evans, K. Stellrecht and S. Harrington

Albany Medical Center, Albany New York 12208

Contact Information:
Susan M. Harrington, PhD
Albany Medical Center
43 New Scotland Ave, MC 22
Albany, NY 12208
harrins@mail.amc.edu
518-262-3506

REVISED ABSTRACT

Acinetobacter species resistant to multiple classes of antibiotics (MDRAc) and carbapenemase-producing *Enterobacteriaceae* (KPC) are nosocomial pathogens that may colonize patients and lead to infections for which treatment options are very limited. These pathogens are of particular importance in high acuity patients such as those in the intensive care unit (ICU). Patients harboring such strains are placed in isolation to prevent transmission. Detection of these pathogens before an infection occurs is a priority. In September 2008 semimonthly surveillance was initiated for all medical and surgical ICU patients. To date 382 perianal swabs have been collected and cultured on 3 media. The media were CHROMagar KPC™ (CHROM), a MacConkey agar plate with 16 ug/ml ceftazidime (MCAZ), and Trypticase soy broth with 2 µg/ml ertapenem (TSB). After overnight incubation a drop from the TSB was placed on MacConkey agar with an ertapenem disc. Sputum specimens (n=197) were cultured only on CHROM and MCAZ. CHROM was designed to detect KPCs in 24 hours; carbapenemase-producing *Enterobacter* and *Klebsiella* appear as blue colonies. Other resistant species are generally buff to white. Media were screened for KPC or MDRAc at 24 and 48 hours by routine methods. KPCs were confirmed with PCR for bla_{KPC}. Twelve perianal cultures and 1 sputum culture were positive for a KPC. Isolates from 12 of 13 cultures grew on all media. Six were *E. cloacae* and 7 were *K. pneumoniae*. MDRAc grew on MCAZ and CHROM from 3 sputum specimens. From perianal specimens 2/4 MDRAc were detected on all 3 media; 1 grew on CHROM and from TSB and 1 from TSB only. At 24 hours false positive cultures were 16 (8%) for MCAZ and 22 (11%) for CHROM from sputum specimens. For perianal specimens the false positive cultures were 44 (11%) for MCAZ, 22 (6%) for CHROM and 57 (15%) for TSB. Importantly, only 1 (0.5%) sputum and 8 (2%) perianal cultures grew colonies that were blue, but not KPCs on CHROM. Six out of 8 colonies were smaller than usually observed for *Enterobacteriaceae*. Incubation for 48 hours increased false positives. Overall, the yield from the 3 media was similar. CHROM was the most specific medium.

INTRODUCTION

Multi-drug resistant pathogens are a significant source of nosocomial infections in hospitals. Screening patients for these bacteria in high risk units can be a useful mechanism for identifying colonized patients. Targeted infection control interventions can then be implemented to reduce the risk of transmission to other patients (1). Recently, guidance for determining if carbapenemase-producing *Enterobacteriaceae* (KPCs) are present in an institution was published. This document includes information on when to perform point prevalence and active surveillance to identify cases and help control the spread of infection once it becomes established in an institution (2).

Currently, there is no FDA approved commercial media or test to detect KPCs or multi-drug resistant *Acinetobacter species* (MDRAc). Landman *et al*. have recommended culturing in Trypticase soy broth to which carbapenem has been added (3). Others have performed PCR and used selective media for recovery from rectal swabs (4). MDRAc are especially problematic because different resistance patterns may be present within the patient population and hospital environment, making development of media with selective antibiotics a challenge. This lack of commercial media requires laboratories to be innovative in preparing in-house media or PCR assays to detect these organisms.

Analysis of the patient population with KPC and MDRAc at the Albany Medical Center showed that most patients had been admitted for some time to an ICU. Active surveillance efforts were therefore focused on the adult MICU and SICU. We collected perianal swabs and sputum specimens for culture of KPCs or MDRAc. These body sites are presumed to be the most likely to be colonized with these species. Three types of media were evaluated. These included CHROMagar KPC™, an in-house prepared MacConkey plate with ceftazidime and Trypticase soy broth with ertapenem.

MATERIALS AND METHODS

Surveillance Population:
In September 2008, semimonthly surveillance of the MICU and SICU at AMC was initiated. Each unit has 17 beds.

Specimens:
Perianal swabs and sputum or tracheal aspirate specimens were collected from consenting patients.

Culture Media:
Media used for surveillance consisted of 3 media prepared in-house. Media were: 1) MacConkey agar with 16 µg/ml of ceftazidime (MCAZ). 2) CHROMagar KPC™ CHROMagar Paris, France (CHROM). Lyophilized medium was rehydrated and supplement was added according to manufacturer's instructions. 3) Trypticase soy broth with 2 µg/ml ertapenem (TSB), generated by placing a 10 µg disc in 5 mls of broth. Solid media were used within 2 weeks of preparation. TSB was prepared 24 hrs prior to use.

Culture Algorithm:
Perianal swabs were plated to all 3 media; sputum specimens were inoculated onto MCAZ and CHROM only. CHROM was protected from light. All media were incubated at 35°C for 20-24 hrs. After overnight incubation, approximately 50 µl TSB was subcultured to a MacConkey agar plate and a 10 µg ertapenem (E) disc was added to the first quadrant. See Figure 1.

Identification of KPC and MDRAc:
MCAZ: All lactose fermenting (LF) colonies were screened with E and meropenem (M) discs using standard Kirby Bauer (KB) methods. Any colony with a zone diameter of ≤ 25 mm for M or ≤ 18 mm for E was considered a potential KPC. Additional tests for identification and confirmation of KPC resistance were performed. Oxidase negative, non-lactose fermenting (NLF) colonies were further screened for MDRAc.

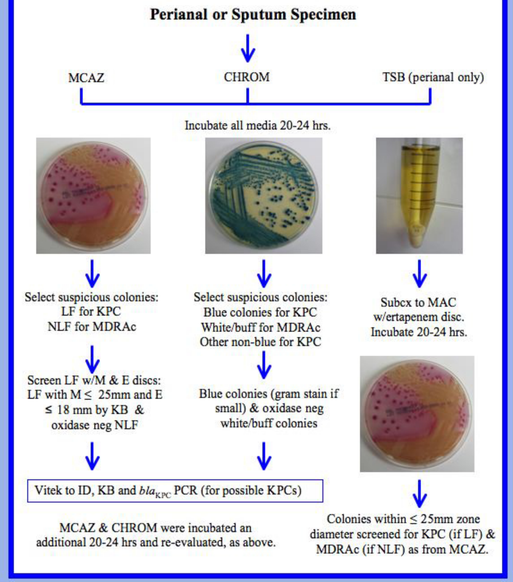
CHROM: All large blue colonies were screened for KPC. Small blue colonies were gram stained to determine if they were gram negative rods before any additional testing was performed. Oxidase negative, white colonies were further screened for MDRAc.

TSB: Any gram negative rod growing on the MacConkey plate from the TSB with an ertapenem zone diameter ≤ 25mm was considered a potential positive. These colonies were screened as from MCAZ above.

All potential KPCs were identified with the Vitek 2 GN card, susceptibility testing was performed by KB and confirmation of KPC was performed by real-time PCR. Oxidase negative, potential MDRAc colonies were screened with Vitek 2 and KB for MDRAc. MDRAc were defined as resistant to 4/5 antibiotic classes including penicillins, cephalosporins, carbapenems, sulfonamides and quinolones.

Real-time PCR: Testing for bla_{KPC} was performed at AMC using a method from Mt. Sinai Hospital, New York City. Primers: KPC721F GGC ACG GCA AAT GAC TAT G KPC 888R GCC AAT AGA TGA TTT TCA GAG C Probes: KPC3 TET- AAG GAT GAC AAG TAC AGC GAT CCT T-B HQ1 KPC 1,2,4 FAM-AAG GAT GAC AAG CAC AGC GAT CCT T-B HQ1. Cycling conditions on the SmartCycler Instrument: 2 minutes at 95°C 35 Cycles: 15 seconds at 95°C; 30 Seconds at 60°C; 10 seconds at 72°C.

Fig. 1 Culture Algorithm



RESULTS

Table 1. Number of cultures positive (%) at 24 hours

KPC:

	MCAZ	CHROM	TSB	Total
Perianal swabs	11 (3%)*	12 (3%)	12 (3%)	382
Sputum	1 (1%)	1 (1%)	N.A.	197

6 *E. cloacae* & 7 *K. pneumoniae*

MDR *Acinetobacter* species:

	MCAZ	CHROM	TSB	Total
Perianal swabs	2 (0.5%)*	3 (1%)	4 (1%)	382
Sputum	3 (3%)	3 (3%)	N.A.	197

*MCAZ was overgrown with other resistant bacteria.

RESULTS

Table 2. Number of Cultures Positive / Number of Cultures Screened at 24 Hours

Perianal swabs:	MCAZ	CHROM	TSB
[a]False pos cxs/total cultures collected	44/382 (11%)	22/382 (6%)	57/382 (15%)
KPC & MDRAc/ total cxs w/colonies screened	13/57 (23%)	15/37 (41%)	16/73 (22%)
KPC pos/total number colonies screened	11/52 LF (21%)	12/20 blue (60%)[b]	12/22 LF (55%)
MDRAc pos/total number colonies screened	2/18 NLF (11%)	3/20 white (15%)	4/59 NLF (7%)

Sputum:	MCAZ	CHROM	TSB
[a]False pos cxs/total cultures collected	16/197 (8%)	22/197 (11%)	N.A.
KPC & MDRAc/ total cxs w/colonies screened	4 /22 (18%)	4/26 (16%)	N.A.
KPC pos/total number colonies screened	1/11 LF (10%)	1/2 blue (50%)	N.A.
MDRAc pos/total number colonies screened	3/12 NLF (25%)	3/27 white (11%)	N.A.

a. False positive culture refers to a culture with any growth that is not a KPC or MDRAc
b. 6 small blue (not gram negative rods), 2 large blue and one violet/pink colony were false positive (non-KPCs) on CHROM

Results at 48 hrs:
No additional KPC or MDRAc.
Additional false positive organisms grew on both MCAZ (9 sputum & 10 perianal cxs) and CHROM (9 sputum & 8 perianal cxs).

RESULTS

1. Over an 8 month period 382 perianal swabs and 197 sputum specimens were collected during semimonthly surveillance in the MICU & SICU at AMC. On average perianal swabs were collected from 75% of patients and sputum from 36% each week.
2. Twelve KPCs were recovered from perianal swabs (3% positive) and 1 from a sputum specimen (1%). MDRAc were recovered from 4 perianal (1%) swabs & 3 sputa (3%).
3. 1 KPC and 2 MDRAc each failed to grow on MCAZ. 1 MDRAc did not grow on CHROM.
4. The number of false positive cultures from perianal specimens was less on CHROM than MCAZ or TSB.
5. For perianal swabs the number of positive cultures compared to the number of colonies screened (% positive) was greatest for CHROM. Percent KPC positive in TSB was similar to CHROM.
6. Many false positives from TSB were *Pseudomonas species* that were easily disregarded with an oxidase test.
7. The number of false positive cultures from sputum specimens was higher for CHROM than MCAZ. These false positives were mainly white colonies that were *Pseudomonas*, non-MDR *Acinetobacter* and *Stenotrophomonas* species.
8. Incubating cultures for 48 hours increased false positives with no additional true positives.

CONCLUSIONS

1. Although the percent of positive cultures was low, the yield of KPCs from perianal swabs was higher than sputum specimens. The yield of MDRAc was better from sputum than perianal cultures.
2. Overall, MCAZ, CHROM and TSB performed similarly for recovery of KPCs from perianal specimens.
3. While the 3 media performed similarly for screening for MDRAc, the small number of isolates recovered limits conclusions. TSB may prove to be a more sensitive medium. Multiple media could be needed to recover MDRAc, depending on susceptibility profiles.
4. For several reasons CHROM is easier to use than TSB and MCAZ for screening KPCs. An intermediate screening step to determine carbapenem resistance is unnecessary for CHROM. TSB requires a subculture step. For perianal specimens both TSB and MCAZ had more false positive colonies, increasing both technical time and turn-around time.
5. Ease of use for detection of MDRAc from sputum is similar for MCAZ and CHROM, but CHROM had more false positive colonies that required screening.
6. CHROMagar can be recommended for screening of perianal swabs and sputum specimens for KPCs.

REFERENCES

1. Kochar S, Sheard T, Sharma R, Hui A, Tolentino E, Allen G, Landman D, Bratu S, Augenbraun M, Quale J. Success of an infection control program to reduce the spread of carbapenem-resistant *Klebsiella pneumoniae*. Infect. Control Hosp. Epidemiol. 2009 30(5):447-52.
2. Lledo,W. M Hernandez, E Lopez, *et al*. Guidance for Control of Infections with Carbapenem-Resistant or Carbapenemase-Producing *Enterobacteriaceae* in Acute Care Facilities MMWR. 2009. 58(10);256-260
3. Landman D, Salvani JK, Bratu S, Quale J Evaluation of techniques for detection of carbapenem-resistant *Klebsiella pneumoniae* in stool surveillance cultures. J Clin Microbiol. 2005 (11):5639-41.
4. Samra Z, Bahar J, Madar-Shapiro L, Aziz N, Israel S, Bishara J. Evaluation of CHROMagar KPC for rapid detection of carbapenem-resistant *Enterobacteriaceae*. J Clin Microbiol. 2008 46(9):3110-1.