Evaluation of RambaCHROM™ Agar: a Comparison of 3 Methods

C-141

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Revised Abstract

- As the prevalence of Carbapenem Resistant Enterobacteriaceae (CRE) rise in medical facilities nationwide, surveillance programs are enacted to control their spread. At the University of Virginia Health System our surveillance program is directed toward *Klebsiella pneumoniae* Carbapenemase producing Enterobacteriaceae (KPC). The CDC provided guidance for laboratory detection of CRE, but the method is laborious and turn-around-times are not optimal. The use of RambaCHROM™ KPC agar (CA) has the potential to resolve these issues. Our study compared 3 culture protocols to determine the most accurate and timely method to detect KPC from perirectal surveillance cultures.
- A total of 588 perirectal specimens were evaluated for the presence of KPC using 3 culture protocols. Two perirectal swabs were collected, with one swab directly inoculated to CA and the other incubated 18-24 hours in Trypticase Soy Broth with a 10ug ertapenem disk (TSB/E) . Samples are then inoculated onto MacConkey agar (MAC) and CA and incubated at 35-37ºC in ambient air. All media were evaluated for possible CRE at 20-24 hours and again at 48 hours post inoculation. Slow to rapid lactose fermenting gram negative rods on MAC and blue or mauve colonies on CA underwent phenotypic testing for KPC using the indirect carbapenemase disk test. The time to detection, number of isolates requiring confirmation and recovery of CRE and KPC from direct inoculation to CA, the TSB/E CA combination and the TSB/E MAC combination were compared.
- A total of 138 isolates recovered from TSB/E MAC protocol required further testing, while 18 from the TSB/E CA and 11 from the direct CA inoculation required further testing. We show the best recovery of CRE and KPC from the TSB/E CA method. Of the suspicious isolates recovered, 13 KPC-producing Enterobacteriaceae were detected by this method, while 8 were detected from both TSB/E MAC and direct CA. All indirect carbapenemase positive isolates were confirmed to be KPC by bla_{KPC} PCR. CA, especially when inoculated after growth in TSB/E and used in conjunction with the indirect carbapenemase disk testing, proves to be an accurate and sensitive method for KPC surveillance.

Introduction

- Carbapenems are a class of broad spectrum beta-lactam antibiotics active against gram positive cocci, gram negative bacilli and anaerobes. Included in this group are Ertapenem, Meropenem, Imipenem and Doripenem. Use of drugs in this class is often reserved for treatment of multi-resistant organisms due to their activity against Gram negative bacilli which produce cephalosporin specific beta-lactamases (AmpC) and Extended Spectrum Beta-Lactamases (ESBL).
- Emerging resistance to carbapenems in the Gram negative bacilli limits treatment options for life-threatening infections involving multi-resistant organisms[1]. While there are a variety of carbapenem resistance mechanisms, the Ambler class A carbapenamases are of particular concern because they are often located on transmissible plasmids. The Enterobacteriaceae are the primary host of the KPC-type Class A carbapenemases, so named due to their discovery in *Klebsiella pneumoniae* in 1996. KPC has since been reported in many other Enterobacteriaceae as well as *Pseudomonas aeruginosa*[2].
- Rapid and accurate detection of Carbapenem Resistant Enterobacteriaceae (CRE) in the clinical microbiology laboratory is necessary for selection of appropriate antimicrobial therapy and implementation of infection control processes to limit spread. In an effort to prevent nosocomial infections, UVa Health System instituted a CRE surveillance program.
- Confirming the presence of CRE in surveillance samples has proven challenging for Clinical Laboratories. Commercial systems and standard *in vitro* susceptibility testing methods are insufficient for accurate detection and identification. The CDC protocol utilizing the Modified Hodge Test proved to be nonspecific in that it could not differentiate mechanisms of carbapenem resistance[3]. These issues prompted our laboratory to seek a more rapid and accurate method for detection and identification of CRE and KPC.

Study Objective

- Utilizing samples acquired for CRE surveillance using a modified CDC protocol, the University of Virginia Health System Clinical Microbiology Laboratory evaluated three culture protocols for CRE detection and KPC confirmation to determine the most accurate, rapid and cost effective method for CRE surveillance.

Materials and Methods

Study Samples
- 588 perirectal swabs routinely collected using BD BBL™ Culture Swab Collection Transport System (Becton Dickinson and Co., Sparks, MD) and submitted to the Clinical Microbiology Laboratory for CRE/KPC surveillance testing.

Media inoculation and incubation
- Media utilized in the study included Trypticase Soy Broth (Remel, Lenexa, KS) with a 10ug ertapenem disk (Becton, Dickinson and Company, Franklin Lakes, NJ) (TSB/E), MacConkey agar (Remel, Lenexa, KS) (MAC), RambaCHROMTM KPC agar (CHROMagar Co., Paris, France) (CA), and Trypticase Soy Agar w/ 5% Sheep Blood (Remel, Lenexa, KS) (BAP).
- Three inoculation methods were utilized.
  - Direct inoculation to CA with swab #1 (Method 1- CA).
  - Swab #2 was placed into 4.5 ml TSB/E and incubated 18-24 hours in ambient air at 35-37°C.
  - 10 µl was inoculated onto MAC (Method 2- TSB/E MAC) and CA (Method 3- TSB/E CA) from the overnight growth in TSB/E.
- All solid media was incubated at 35-37°C in ambient air.

Media interpretation
- MAC was examined for the presence of slow to rapid lactose fermenting Gram negative bacilli at 20-24 hr and again at 48 hr.
- CA (direct and subcultured from TSB/E) was examined for colony growth and color formation at 20-24 hr and again at 48 hr.
  - Blue colonies on CA are associated primarily with carbapenemase-producing *Klebsiella pneumoniae*, but other Enterobacteriaceae may also produce this color.
  - Mauve colonies on CA are associated with carbapenemase-producing *Escherichia coli*.
- All organisms suspicious for carbapenemase production were subcultured to BAP and incubated 18-24 hr prior to identification, susceptibility testing, and KPC confirmatory testing.

KPC Confirmatory testing
- The indirect carbapenemase disk method[4] was used to determine the presence or absence of KPC production in all suspicious isolates.
  - Media and reagents include Mueller Hinton agar (Remel, Lenexa, KS) (MHA) and 10µg meropenem disk (Becton, Dickinson and Company, Franklin Lakes, NJ) and Iris-EDTA disk (Becton, Dickinson and Company, Franklin Lakes, NJ).
  - Actively growing *E. coli* ATCC 25922 diluted to 0.5 McFarland is inoculated to MHA as a lawn and allowed to absorb into medium.
  - A 10µg meropenem disk is placed on the agar surface.
  - A tris-EDTA disk is inoculated with a loop full of test organism and placed organism side down, near the meropenem disk (1-2 mm).
  - Inoculated medium is incubated at 35-37°C in ambient air for 24 hr.
  - An alteration in the zone of inhibition around the meropenem disk confirms the presence of KPC production.
- bla_{KPC} PCR was performed as previously described by Mathers et al[5].

Organism identification and Antimicrobial susceptibility testing
- Reagents include bioMerieux GN ID and AST GN24 cards(bioMerieux, Durham,NC)
- All isolates were assayed on the Vitek II system per manufacturer's protocol.

Results

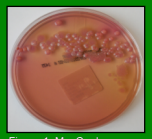

Figure 1: MacConkey agar with mixed morphologies of Gram negative bacilli from TSB/E MAC method

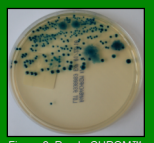

Figure 2: RambaCHROM™ KPC agar with 2 morphologies of CRE from TSB/E CA method

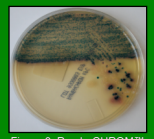

Figure 3: RambaCHROM™ KPC agar with CRE and *Pseudomonas sp.* from CA direct inoculation method

- Percentage of specimens (n=588) and number of isolates requiring further evaluation and confirmatory testing by the indirect carbapenemase disk test with the estimated tech time associated with set up, test result evaluation and documentation (assuming 5 min hands on time per isolate to complete process).
  - 15.5% (91 specimens & 138 isolates) using the TSB/E MAC method; estimated additional tech time for confirmatory testing is 11.5 hrs (Figure 1).
  - 2.9% (17 specimens & 18 isolates) using the TSB/E CA method; estimated additional tech time for confirmatory testing is 1.5 hrs (Figure 2).
  - 1.7% (10 specimens & 11 isolates) using the CA direct inoculation method; estimated additional tech time for confirmatory testing is 55 min (Figure 3).

Results

Method	Specimens with growth suggestive of CRE (number of isolates)	Number of isolates KPC positive by indirect carbapenemase disk test	Number of KPC positive isolates recovered only from the method listed	Number of isolates positive by bla_{KPC} PCR
TSB/E MAC	91 (138)	8	1	8
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- A total of 15 KPC-producing Enterobacteriaceae were identified during this study.
  - A variety of species within the Enterobacteriaceae were identified, including *Enterobacter* sp, *Klebsiella pneumoniae*, *Klebsiella oxytoca* and others (Figures 1-3).
  - Of the 18 CRE isolates recovered from the TSB/E CA method, 17 were identified at 24 hr incubation. The single isolate identified at 48 hr incubation was also confirmed to be a KPC-producing Enterobacteriaceae.
  - All 15 isolates confirmed to be KPC-producing Enterobacteriaceae were positive by bla_{KPC} PCR.
    - The TSB/E CA method identified the greatest number of CRE confirmed to be KPC-producing Enterobacteriaceae (13).
    - The isolate identified by TSB/E MAC only, produced blue colonies consistent with CRE upon subculture to CA.
- No mauve colonies consistent with carbapenem resistant E coli were identified in the course of this study

Conclusions

- The use of chromogenic media has proven utility in organism identification from urine culture, MRSA surveillance, and yeast culture. We show utility for the use of chromogenic media for identification of CRE and KPC-producing Enterobacteriaceae from perirectal surveillance samples as well
- A variety of organisms will grow on RambaCHROM™ KPC agar, including *Pseudomonas*, *Stenotrophomonas*, *Acinetobacter* and gram positive cocci. With minimal training and experience, technologists easily adapt to the color and colony characteristics of CRE on RambaCHROM™KPC agar
- Three clinical specimens (6 isolates) grew blue colonies consistent with CRE and identified as Enterobacteriaceae with elevated MIC values to Ertapenem, but were negative for KPC production by both indirect carbapenemase disk test and bla_{KPC} PCR, therefore carbapenem resistance was likely due to a mechanism other than a Class A KPC-type Carbapenemase
- The addition of RambaCHROM™ KPC agar to our surveillance program significantly decreased the number of isolates requiring evaluation and confirmatory testing as compared to the modified CDC protocol (TSB/E MAC) currently in use at our institution. This offered a reduction in tech time required to provide results, while decreasing turn-around-times and improving recovery of CRE in surveillance samples
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References

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- 3. Moland, E. "Newer B-Lactamases: Clinical and Laboratory Implications, Part II" Clinical Microbiology Newsletter.30.11 (2008).81.
- 4. Thomson,K.L., "Newer Beta-Lactamases Workshop" SEACM 2007.
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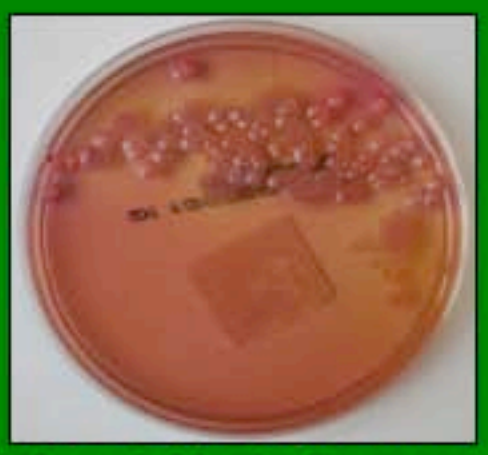

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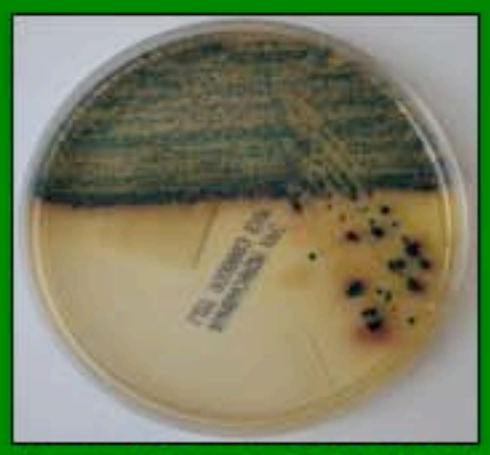

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