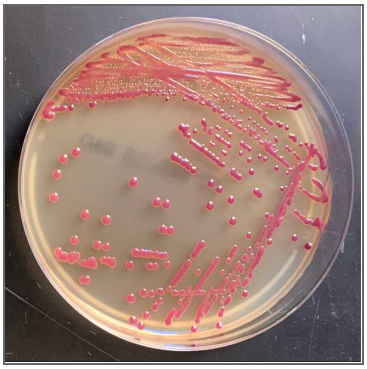

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CPE Culture from Companion Animal Rectal Swabs or Feces

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DISCLAIMER

Reference to any commercial materials, equipment, or process does not in any way constitute approval, endorsement, or recommendation by the Food and Drug Administration.

ABSTRACT

Carbapenemase-producing Enterobacterales (CPE) are one of the most urgent threats to human healthcare globally. Our study aimed at optimizing the chromogenic agar method of screening for CPE with and without selective enrichment to isolate CPE from animal feces. The selective enrichment step did not result in any increased recovery of CPE from companion animals with NDM-5 *E. coli*, which suggests that enrichment broth may not be necessary for outbreak surveillance testing. Therefore, the selective enrichment step is optional, however, this method may not be generalizable to the detection of all types of CPE in fecal specimens from companion animals.

IMAGE ATTRIBUTION

https://www.vet.upenn.edu/veterinary-hospitals/ryan-veterinary-hospital/services/diagnostic-laboratories/create/labcreate

GUIDELINES

Samples should be processed within 72 hours of receipt at the laboratory.
Rectal swabs and feces should be refrigerated at 4-8 °C until processed.
All plates should be stored at 4-8°C in a container that protects the agar from light exposure.
Selective enrichment is optional.

Protocol status: Working
We use this protocol and it's working

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PROTOCOL integer ID: 92826

Keywords: Carbapenemase-producing Enterobacterales, Carbapenem-resistant Enterobacterales

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MATERIALS

Control Strains:

- Negative control: ATCC 29522 E. coli
- Positive control(s): NCTC 13440 K. pneumoniae (VIM), NCTC 13442 K. pneumoniae (OXA-48), NCTC 13476 E. coli (IMP), BAA-1705 K. pneumoniae (KPC), BAA-2469 E. coli (NDM)

Media

- CHROMID Carba agar, bioMérieux Catalog #414012
- Tryptic soy broth, Hardy Diagnostics Catalog #R30 (optional, only if performing enrichment)

Supplies

- 1-µL inoculation loops
- 10-µL inoculation loops (optional, only if performing enrichment)
- 10-µg meropenem Kirby-Bauer disc, Fisher Scientific Catalog #B4331703 (optional, only if performing enrichment)
- Sterile forceps (optional, only if performing enrichment)

Equipment needed:

- Incubator capable of maintaining 35 ± 2°C

CPE Culture from Companion Animal Rectal Swabs or Feces

1 If starting with feces, dip a cotton tipped applicator into the specimen. The swab does not have to be fully covered with feces, but it should be visibly inoculated. If starting with a rectal swab, proceed to step 2.

2 Using the inoculated swab, streak the first quadrant of the Carba plate.

3 Finish streaking the remainder of the quadrants on the plates using a 1-µL inoculation loop.

4 Using sterile forceps, add a 10-µg meropenem disc to 5 mL of tryptic soy broth (TSB-MEM).

5 Break off the swab or add a pea-sized amount of feces into the TSB-MEM broth.

6 Incubate both the primary plate and inoculated TSB-MEM at 35 ± 2 °C for 18-24 hours.

7 After 18-24 hours, observe the primary Carba plate for suspect colonies following manufacturer's instructions.

7.1 For CHROMID Carba plates, pink-red colonies are presumed to be CPE *E. coli* while blue-green/grey colonies are presumed to be CPE *Klebsiella*, *Enterobacter*, *Serratia*, or *Citrobacter.* White, yellow, or tan colonies are presumed to be non-*Enterobacterales*.

8 Using a 10-µL inoculation loop, subculture the inoculated TSB-MEM broth to a secondary Carba plate.

9 Incubate the secondary Carba plate at 35 ± 2 °C for 18-24 hours.

10 After 18-24 hours, observe the secondary Carba plate for suspect colonies following manufacturer's instructions.

11 After suspect colonies have been cultured, identification can be performed by subculturing the colony to a blood agar plate then identifying the organism following standard laboratory procedures. Confirmation

of carbapenemase production can be performed by modified carbapenem inactivation method (mCIM), EDTA-modified carbapenem inactivation method (eCIM), CarbaNP, NG-Test CARBA 5, CARBA-R PCR, or a lab-developed PCR.