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Journal
American Journal of Infection Control, 49(1)

ISSN
0196-6553

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Publication Date
2021

DOI
10.1016/j.ajic.2020.05.023

Peer reviewed

Detection of carbapenem resistant enterobacteriace from fomite surfaces

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Accurately identifying carbapenem resistant enterobacteriace (CRE) from fomites is critical for infection control practices, research, and assessing patient risk. We compared a commercial CRE agar intended for patient use with a modified MacConkey agar. We found that our modified MacConkey agar was more selective at identifying CRE from environmental sources.

Carbapenem-resistant enterobacteriaceae (CRE) are pathogens in health care facilities associated with high morbidity, mortality, and limited treatment options.[1] CRE can be transmitted via contaminated fomites, emphasizing the importance of environmental contamination as a potential source of facility amplification.[2] Accurate detection of environmental contamination is a critical component to understanding the CRE burden and transmission. However, methods to detect CRE from environmental sources are not standardized, limiting our ability to evaluate this clinically relevant issue. We describe our experience comparing 2 selective medias to identify CRE from fomites.

We collected environmental samples using Sponge-Sticks (3M, St. Paul, MN) from high-touch fomites at 3 nursing homes (NH) that participated in Project PROTECT, a pilot study of topical decolonization to prevent multidrug-resistant organism colonization.[2] Swabs were collected from ten most common contaminated fomites (light switch, bed rails, tables, phones, remotes, call buttons, toilet flush handle, sink handles, bathroom rails, and door knobs) from 356 NH resident rooms (1,780 swabs total).[2] Within 6 hours of collection, swabs were enriched in liquid Tryptic Soy Broth for 10-12 hours.[4]

We compared 2 selective medias for CRE identification. The first involved HardyCHROM CRE plates (Hardy Diagnostics, Cincinnati), plates that are intended for CRE identification from patient, not environmental samples. The second involved modified MacConkey agar (m-MacConkey). The use of antibiotic impregnated plates is not new and has been shown to be effective for screening for CRE.[3] This m-MacConkey (Hardy Diagnostics, Cincinnati) was supplemented with 1 mg/mL meropenem by mixing 26.245 grams of MacConkey agar powder and 1,000 mL of deionized water, autoclaving, and cooling. Before the agar solidified, 100 mL of meropenem (10 mg/mL) was added to the agar, mixed, and then pipetted into Petri dishes.[4] After being made the plates were stored at 4°C until used (≤72 hours). Environmental samples were streaked and incubated overnight at 37°C.

Stock organisms were used to validate each batch of HardyCHROM CRE plates and m-MacConkey with: Klebsiella pneumoniae carbapenemase producing ATCBAA-1705 (KPC+), Klebsiella pneumoniae carbapenemase nonproducing ATCBAA-1706 (KPC-), Methicillin resistant Staphylococcus aureus ATCC-43300 (MRSA), and Klebsiella pneumoniae Extended Spectrum Beta-Lactamase ATCC-700603 (ESBL).

Blue colonies on HardyCHROM CRE plates were defined CRE positive, as per manufacturer's instructions. On m-MacConkey, pink colonies (lactose fermenters) were defined as likely CRE positive, but all organisms growing were evaluated. To confirm carbapenemase production on positive samples from m-MacConkey, a Modified Hodge Test (MHT) was performed to detect true carbapenemase producing organisms (CP-CRE) using published guidelines.[1] At the time of the study MHT was considered to be a standard procedure, but is no-longer considered the test of choice.[4] MHT positives were further identified via biochemical analysis using the VITEK 2 (BioMerieux) automated system at Harbor-UCLA Clinical Microbiology Laboratory. Of the 1,780 fomites tested, 176 of 1,780 (9.9%) screened CRE positive
by HardyCHROM CRE plates. Given that there were a high number of positives from fomites per room, yet a low prevalence of CRE colonization of SNF residents (<1%),2 we were concerned about false positives. Given the concerns for false positives, we went back to stock cultures for the positive CRE organism identified by Hardy-CHROM CRE plates and compared them to m-MacConkey. On m-Mac-Conkey plates, 27 of 176 (15%) isolates screened positive for carbapenem resistance, 8 (30%) of which were confirmed to be CPCRE via MHT. In addition, 76 (43%) of the HardyCHROMTM CRE isolates (representative set) were sent for testing at University of California, Irvine Clinical Microbiological Laboratory. This Laboratory chose the first 20 isolates and then a subsequent random sample of 18 isolates for standardized clinical microbiologic testing for species and susceptibility validation, including use of the VITEK 2 system. Of these 38 isolates, 2 (5%) were CRE (Klebsiella pneumoniae (n = 1), Enterobacter aerogenes (n = 1)), and 6 (15%) were non-carbapenemase producing Acinetobacter baumannii (n = 6)) (Fig 1). The remaining isolates (n = 30) were carbapenem susceptible.

Eight of the 27 CP-CRE isolates from the m-MacConkey plates were identified by the VITEK 2 system as CRE, K. pneumoniae (n = 7), E. cloacae (n = 1) at Harbor-UCLA Clinical Microbiology Lab. (Fig 1)
The remaining 19 (70%) were found to be non-carbapenemase producing organisms (nCP-CRO) based on MHT. Species included Pseudomonas spp. (n = 5), Aeromonas spp. (n = 1), Stenotrophomonas maltophilia (n = 1), and the remaining were not identified with a high level of confidence using the VITEK 2 system (unknown, n = 12).

Accurate measurement of CRE environmental contamination is important for infection control practices, research, and assessing patient CRE acquisition risk. HardyCHROM CRE plates are practical and simple for evaluating patient samples, but we found their use for environmental surveillance problematic. Commercial HardyCHROM CRE plates had a high false positivity rate from environmental samples and lacked selectivity for identifying CP-CRE or nCP-CRO from environmental sources. Of note, HardyCHROM CRE plates are neither approved nor designed to identify environmental CRE. The reason for the poor performance for identification of environmental on Hardy-CHROM CRE is unclear. Conversely, m-MacConkey plates are able to select both CP-CRE and nCP-CRO from the same samples and had fewer false positives (P<.01).

In conclusion, the use of selective media to identify CRE from the environment continues to pose significant challenges. We share our experience with HardyCHROM and m-MacConkey to guide researchers interested in environmental contamination about the challenges in using readily available diagnostic agars outside of their recommended use. Use of m-MacConkey maybe beneficial to detect clinically relevant CRE and nCP-CRO fromenvironmental samples. Further data is needed to determine a best method for environmental sampling to help clarify risks with carbapenem resistant organisms and colonization of the environment. We understand that MHT is no longer a laboratory standard to identify CRE, but at the time of the study MHT was considered acceptable. Nowadays, molecular methods should be used to detect carbapenemase in a suspected CRE isolate. The distinction and importance
of carbapenemase production has been de-emphasized by laboratory standards groups, as evidence by Clinical Laboratory Standards Institute lowered breakpoints to detect clinically relevant CRE organisms regardless of CP production.[5,6] Our research highlights limitations of commercial chromogenic agars for environmental isolation of CRE. Future studies may wish to examine the accuracy of other commercial agars for isolation of CRE from environmental samples.

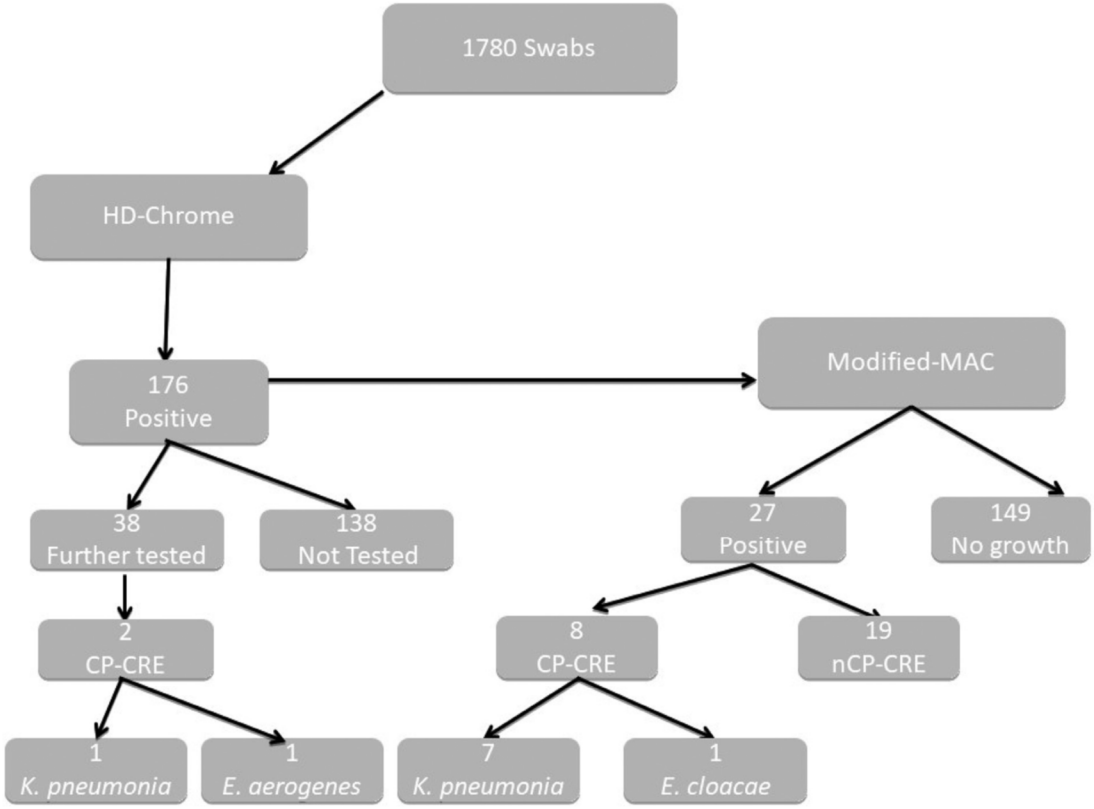

Fig 1. Carbapenemase resistant enterobactericae true (CP-CRE) confirmation. HD-Chrome, commercial HardyChrome plates; Modified-MAC, Modified MacConkey Agar; CP-CRE, carbapenemase resistant enterobactericae producers; nCP-CRE, carbapenemase resistant enterobactericae nonproducers.

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Conflicts of interest: None to report.