Detection of High Rates of Multidrug Resistant Bacteria Carriage with Extended Spectrum β-Lactamases (ESBLs), Carbapenem Resistant *Enterobacteriaceae* (CRE) and *Clostridium difficile* Using the Copan FecalSwab®

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Background

Long-term acute care hospitals (LTACHs) are health care facilities that admit complex patients with acute care needs and multiple co-morbidities which place them at risk of colonization with Gram-negative Multidrug-Resistant Organisms (MDROs) eg. Extended Spectrum β-lactamases (ESBLs) and Carbapenem Resistant *Enterobacteriaceae* (CREs). There are few data available on active screening for multiple MDRO co-colonization with *Clostridium difficile* in LTACHs. Such data may help direct infection control interventions in an effort to decrease MDRO infection rates.

Liquid based transport systems with flocked swabs improve detection of pathogens compared to standard fiber wound swabs. The Copan FecalSwab® Collection, Transport and Preservation System (Copan Diagnostics, Murrieta, CA) consists of a nylon flocked swab in 2 mls of modified Cary-Blair medium that is designed for the recovery and detection of enteric pathogens. We conducted a point-prevalence study of CREs, ESBLs, and *Clostridium difficile* (*Cdif*) carriage among LTACH patients and compared the Copan FecalSwab® (Figure 1) to a rayon swab for rectal carriage of ESBLs, CREs and *Cdif* using culture and PCR. The goal was to determine MDRO carriage rates and to isolate and prevent spread of MDROs.

Materials and Methods

Specimens:

- The study was conducted in two Long-term Acute Care Hospitals in the Chicagoland area in April & September 2017 respectively as part of an infection prevention program.
- Two rectal specimens were collected together for detection of CRE, ESBL, and *Cdif*:
  - 1 double headed Rayon swab in Liquid Amies (BBL, Becton Diagnostics).
  - 1 Copan FecalSwab® (flocked swab in 2mls of Cary-Blair Medium).

C difficile detection using Cepheid Xpert® *C. difficile/Epi* PCR:

- 400uL of sample from the FecalSwab transport tube was transferred into the elution reagent tube and vortexed at high speed for 10 seconds.
- The entire elution reagent was then transferred to the specimen chamber in the GeneXpert® cartridge.
- Real-time PCR was performed by using GeneXpert® Dx instrument (Cepheid, Inc., Sunnyvale, CA) in accordance with the Cepheid Xpert® C. difficile/Epi assay's package insert.

CRE detection using Cepheid Xpert® Carba-R PCR:

- Another 400uL of sample from FecalSwab transport tube was transferred into the elution reagent tube and vortexed at high speed for 10 seconds .
- The contents of the cartridge were transferred using the pipette provided. The sample reagent was aspirated up to the mark on the pipette (approximately 1.7 mL) and then transferred into the specimen chamber of the Xpert Carba-R cartridge.
- Real-time PCR was performed by using GeneXpert® Dx instrument (Cepheid, Inc., Sunnyvale, CA) in accordance with the Cepheid Xpert® Carba-R assay's package insert.

Methods cont'd

Culture Testing:

ESBL and CRE Culture using Copan Fecalswab®:

- A10uL sample from the Copan Fecalswab transport tube was plated directly onto one-half of a HardyCHROMagar™ ESBL plate (Hardy Diagnostic, Santa Maria, CA).
- Plates were incubated at 33-35˚C for up to 24 hours.
- After incubation, plates were examined for pink (*E. coli*), blue (*Klebsiella spp*) and tan colonies with a brown halo (*Proteus spp*) and subcultured to a Blood Agar Plate (BAP) for isolation. (Figure 2)
- Identification of suspected organisms were performed using MALDI-TOF (BD Bruker) and susceptibility testing performed by Kirby-Bauer disk test following CLSI guidelines.
- The list of an antibiotic-containing disks tested are shown in Table 1.
- Phenotypic testing for ESBL detection was performed using Cefotaxime and Cefotaxime-clavulanate disks and Ceftazidime and Ceftazidime-clavulanate disks as per CLSI recommendation.
- Any organism non-susceptible to a carbapenem, was considered a probable CRE.

ESBL and CRE Culture using Double-headed BBL Rayon swab:

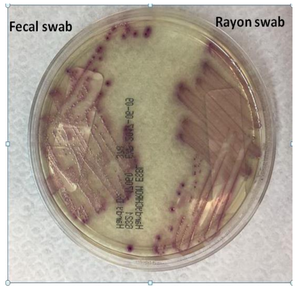
- One of the rayon rectal swabs was simultaneously plated onto the second-half of the HardyCHROMagar™ ESBL plate .
- Plates were incubated and examined as above and any growth was subcultured to a Blood Agar Plate (BAP) for isolation and identification using MALDI-TOF.
- Presence of growth was also compared between Copan Fecalswab and rayon swab.

***C difficile* Culture:**

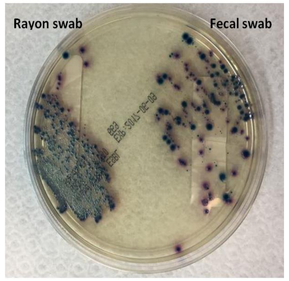
- Culture confirmation was performed for all PCR positive *C. difficile* specimens and an equal number of negative samples.
- Both rectal swabs were cultured using CCFA-HT agar (Anaerobes systems, Morgan Hill, CA).
- Plates were incubated in an anaerobic jar for 5 days at 37 ˚C.
- Presumptive *Cdif* colonies, yellow, ground-glass or large colonies with irregular edges underwent Gram stain and aerotolerance testing.
- Growth of spore forming, anaerobic Gram positive rods that were PRO disk positive (Hardy Diagnostics, Santa Maria, CA) were considered *Cdif* culture positive.

Figure 1. Copan FecaSwab®

Figure 2. HardyCHROM™ ESBL plates



E. coli



Klebsiella spp.

Table 1. List of antibiotic-containing disks used for susceptibility testing

Aztreonam	Ertapenem
Ceftazidime	Meropenem
Ceftriaxone	Cefoxitin
Cefotaxime	Cefoxitin
Cefepime	Tobramycin
Cefotaxime-clavulanate	
Ceftazidime-clavulanate	

Results

- 119 patients were eligible for the study and a total of 107 patients with paired rectal swabs were included in the final data analysis .
- Mean age of patients was 59 years (range: 24-90) and 45 out of 75 (60%) patients were male .
- 9 out of 107 (8%) had FecalSwab positive for *Cdif* by PCR.
  - 7 out of 9 PCR positive specimens were also *Cdif* culture positive.
  - The 2 discrepant specimens were tested using a second PCR (Roche Cobas *C difficile* test); both specimens tested positive.
- 46 out of 107 (43%) patients had at least one MDRO
  - 23 FecalSwab specimens (21.5%) patients had CRE rectal colonization.
    - They were all bla_{KPC} PCR positive
  - CRE culture was positive for 20/23 specimens and concordant for both swab types.
    - 2 specimens were positive after repeat culture using enrichment broth incubated overnight; overall 22/23 were culture positive
    - Results of FecalSwab culture vs PCR are shown in Table 2
  - 23 FecalSwab specimens (21.5%) were ESBL culture positive.
    - 21 /23 were also positive using rayon swab.

Table 2. CRE culture and PCR Results

Testing Methods	Number of Positive
CRE Culture using Copan FecalSwab®	17 *K.pneumoniae* 1 *K.pneumoniae* and *E.aerogenes* 1 *E. cloacae* *2 E. aerogenes* *1. E. coli*
Xpert Carba-R	23 bla_{KPC}

Conclusion

- There is a high rate of Gram-negative MDRO and *Cdif* carriage (46%) in LTACH facilities.
- The Copan FecalSwab allows for a single specimen to be collected for both culture and molecular testing.
- The Copan FecalSwab has a high sensitivity and specificity for rectal detection of CREs, ESBLs and *C. difficile* and can be used as part of an infection control surveillance program.