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Viability of bacterial enteropathogens in fecal samples in the presence or absence of different types of transport media

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ABSTRACT

Transport media are recommended to improve the sensitivity of fecal culture, but there are limited published data comparing bacterial viability in feces stored with or without transport media. In this study, recovery of bacteria from culture-positive feces after 7 days of storage was assessed under the following conditions: without transport media (w/oTM); with FecalSwab™ Transport and Preservation Medium (FSTM); and with modified Cary–Blair (mCB). All Shiga toxin–producing *E. coli* (STEC) positive specimens ($n = 23$) and ≥97.5% of *Salmonella*-positive specimens ($n = 40$) remained positive under all conditions. *Campylobacter* ($n = 41$) was isolated from 82.9% of feces stored in mCB, 68.4% in FSTM, and 70.7% w/oTM; *Shigella* ($n = 14$) 85.7%, 78.6%, and 78.6%; and *Yersinia* ($n = 16$) 93.8%, 87.5%, and 81.3%, respectively ($P = 0.076$, Cochran's Q). Transport media were not required for STEC or *Salmonella.* mCB may be better than w/oTM or FSTM for other pathogens, but an evaluation with a larger number of specimens is required.

1. Introduction

Microbiology best practices for stool culture recommend transporting feces in Cary–Blair (CB) or modified CB (mCB) media to improve the success of culture (Baron 2011; Humphries and Linscott 2015; Association of Public Health Laboratories 2016; Pillai 2016; Berenger et al. 2017). mCB is preferred because it is superior compared to CB for the recovery of *Campylobacter* by culture (Wang et al. 1983). Although these recommendations are "standard" practices in clinical/medical microbiology, fecal transport media are not universally used by laboratories due to short transportation times and financial constraints, and the same fecal specimen may be required for other laboratory tests in which feces in transport media have not been validated (e.g., culture-independent *C. difficile* testing). Furthermore, if one reviews the references cited to support recommendations that mCB or CB be used for fecal culture specimens, none of these citations have data that directly compare feces stored without transport media (w/oTM) to feces stored with transport media (Cary and Blair 1964; Luechtefeld et al. 1981; Wells and Morris 1981; Wang et al. 1983). This is also true of published data assessing the performance of new fecal transport media such as Fecal Swab™ Transport and Preservation Medium (FSTM) [Copan Diagnostics Inc., Murrieta, CA], which has also not been directly compared to the recommended standard (mCB or CB) (Hirvonen and Kaukoranta 2014).

As many laboratories move to culture-independent tests (CIDTs) for the diagnosis of bacterial gastroenteritis, the use of transport media must be reconsidered for 2 reasons. The first consideration is that not all CIDT assays have been validated using transport media. The second is that CIDT still requires culture to be performed (Association of Public Health Laboratories 2016; Berenger et al. 2017; Marder et al. 2017; Shane et al. 2017), but time to culture inoculation may be delayed because culture might be deferred until a positive CIDT result is obtained, or culture is referred to a different site such as the public health laboratory. The Association of Public Health Laboratories in the United States of America has therefore recommended the use of mCB and the Canadian Public Health Laboratory Network recommends transport media for fecal specimens if long turnaround times are expected (Association of Public Health Laboratories 2016; Berenger et al. 2017).

In the context of widespread implementation of CIDT for bacterial gastroenteritis and the lack of proper comparisons in published validations of fecal transport media, we performed an evaluation assessing the

Abbreviations: mCB, Modified Cary–Blair; FSTM, FecalSwab™ Transport and Preservation Medium; STEC, Shiga toxin–producing *E. coli*; CIDT, Culture-independent diagnostic testing.

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viability of bacterial agents of gastroenteritis in clinically positive feces stored in the absence of transport media as well as in 2 commonly used fecal transport media (mCB and FSTM).

2. Methods

2.1. Clinical positive feces

Clinical fecal specimens which tested positive by culture for *Aeromonas, Campylobact*er, *Salmonell*a, *Shigella,* Shiga toxin–producing *Escherichia coli* (STEC), or *Yersinia* at Alberta Public Laboratories, Calgary, Canada (the Alberta Provincial Laboratory for Public Health (ProvLab) and Calgary Hub (formerly Calgary Laboratory Services) (Calgary, Canada) and DynaLIFE Laboratories (Edmonton, Canada) were stored at 4 °C until transport on ice packs to ProvLab. All fecal specimens were collected w/oTM.

2.2. Viability comparison

At ProvLab, stools were mixed well with sterile sticks if solid or vortexed for 10 s if liquid. A "pea-sized" amount (approximately 200 μL) was transferred into 3 different polypropylene tubes containing: 1) 2 mL of mCB (Dalynn Biologicals Inc., Calgary, AB); 2) 2 mL of FSTM; or 3) fecal specimen w/oTM. At day 0 (experiment start date), a sample from each of the 3 conditions was cultured for the organism as previously identified. All 3 tubes were subsequently refrigerated at 4–8 °C, and a sample was taken for culture at day 2, 4, and 7. At each time point, mCB and FSTM medium tubes were vortexed for 10 s, and 3 drops (~100 μL) from a transfer pipette were inoculated onto culture agar and 6 drops (~200 μL) into a broth for enrichment if required. Fecal specimen stored w/oTM was applied over a dime-sized area on agar using a sterile cotton swab and streaked for isolation. Where broth enrichment was required, a "pea-sized amount" of solid feces or 6 drops of liquid feces (~200 μL) from a transfer pipette was inoculated. Culture media specific to each organism and basic identification steps are shown in Table 1. All plates were incubated at 35 °C except for *Campylobacter*, which was incubated at 42 °C in microaerophilic conditions. API® (bioMérieux, St Laurent, QC) was used for identification of the organisms when applicable.

Specimens were excluded from the final analysis if at least 1 of the following criteria was not met: 1) If the pathogen was not recovered on day 0; 2) if 2 or more time points were missing over the period of 7 days; 3) if the 7-day time point was missed.

2.3. Statistical analysis

IBM SPSS Statistics v 25.0.02 was used to determine if there was a statistically significant ($P < 0.05$) difference in the viability of pathogens between feces storage conditions

2.4. Ethics

The work described in this manuscript involved the secondary use of anonymized samples; therefore, ethics review was not required as defined in Article 2.4 of the Government of Canada Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans (2014).

3. Results

The viability of the organisms stored at 4 °C over a period of 7 days is shown in Table 2. The recovery of STEC ($n = 23$) and *Salmonella* ($n = 40$) in feces w/oTM and both types of media was 100% and 97.5%, respectively (Table 2). As for *Campylobacter* ($n = 41$), *Shigella* ($n = 14$), and *Yersinia* ($n = 16$), the viability decreased in feces w/oTM and also in both types of media over 7 days, but a higher proportion of viable organisms were detected in mCB after 7 days as compared to organisms in FSTM or feces w/oTM (Table 2). Less than 90% of the isolates could be recovered from feces w/oTM after 2 days for *Campylobacter,* 4 days for *Shigella,* and 7 days for *Yersinia,* and similar findings were observed in FSTM transport media. The enhanced recovery of *Campylobacter, Shigella*, and *Yersinia* with mCB was not statistically significant ($P = 0.076$, Cochran's *Q* test at 7 days).

Aeromonas was difficult to recover due to not only reduced viability but also overgrowth of the bacteria from the gut flora on the blood agar used for the recovery of these organisms. Therefore, the viability of *Aeromonas* was not analyzed.

Although we were not able to recover 100% of the bacteria identified by the laboratory performing the initial culture, the proportion of feces with viable organisms upon culture for the study gives an estimate for the viability of each pathogen in feces w/oTM. The proportion of specimens with viable organism cultured at time zero for the study is as follows: *Campylobacter* ($n = 42/66$, 63.6%), *Shigella* ($n = 16/19$, 84.2%), *Yersinia* ($n = 17/21$, 80.9%), *Salmonella* ($n = 46/48$, 95.8%), and STEC ($n = 24/24$, 100%) (Table 3). As per the exclusion criteria, 1 *Campylobacter,* 2 *Shigella*, 1 *Yersinia,* 6 *Salmonella*, and 1 STEC, positive specimens that grew at time zero for the study were not analyzed as a part of the 7-day viability assessment in Table 2.

The time from collection was not available for all feces received, but for specimens with known collection times, the average time from collection to culture for the study was 7 to 15 days (Table 3). There were a large number of *Campylobacter*-positive specimens that failed to grow at day 0, but no apparent difference was observed in the time from collection to culture for the study for those specimens that were positive ($n = 42$) (average 9; median 6; first–fourth quartiles = 4.8, 6.5, 9.1, 41.8); range 3–42 days) and those that were not ($n = 24$) (average 10; median 6;first–fourth quartiles = 5.3, 6.4, 10.6, 42.9; range 3–43 days). The 17 *Yersinia* specimens that were positive at the time of culture for the study had an average time from collection to culture for study of 5 days (median 5; first–fourth quartiles = 4, 4.5, 6.5, 8; range 3–8); those that were negative at time of culture for the study were collected 7, 7, 10, and 21 days prior to receipt. The 2 *Salmonella*

Table 1
Media and minimal identification used to identify viable organism.

Organism	Culture	Subculture	Identification
Aeromonas spp.	BAP	N/A	Hemolysis, Oxidase, Basic ID Media
E. coli O157	CHROMagar™ O157	N/A	Mauve colony + O157 Antisera
STEC (non-O157)	CHROMagar™ STEC	CHROMagar™ STEC	Mauve colony + QUIK CHEK™ Shiga Toxin
Campylobacter	CBF	N/A	Colony morphology, Gram stain
Salmonella	MAC, HEK, m-Sel	m-Sel to SS	Basic ID Media, Poly-O Antisera
Shigella	MAC, HEK, m-Sel	m-Sel to SS	Basic ID Media, Poly-O Antisera
Yersinia	CIN	N/A	Colony morphology, Basic ID Media

All incubation times were 24 h except for *Campylobacter*, which was 48–72 h. Identification was performed at day 0 and 7 days or the last day in which a suspect colony was identified. For other days, colony morphology was used to identify growth. Basic ID Media included Triple-Sugar Iron Agar, Sulfide Indole Motility (ProvLab), Urea and MacConkey-Crystal violet agar (MAC) as appropriate for the pathogen. CHROMagar™ media from CHROMagar Microbiology (Paris, France) included tellurite for O157 (as per manufacturer's instructions). BAP = blood agar plate, CBF = *Campylobacter* Blood Free Agar, m-Sel = mannitol–selenite broth, SS = *Salmonella Shigella*, WB = Wilson–Blair, CIN=Cefsulodin–Irgasan–Novobiocin Agar. All media from Dalynn Biologicals unless otherwise stated. Shiga Toxin QUIK CHEK™ was made by TechLab (Blacksburg, VA).

Table 2
Proportion of fecal specimens with viable bacteria after storage without transport media, in FSTM, or in mCB stored at 4–8 °C for 2, 4, and 7 days.[a]

		Viability at different time points (%)		
	No. of specimens included for data analysis	$T = 2$ days No. viable/no. analyzed (%)	$T = 4$ days No. viable/no. analyzed (%)	$T = 7$ days No. viable/no. analyzed (%)
Campylobacter	41			
Feces		36/41 (87.8)	36/41 (87.8)	29/41 (70.7)
FSTM		37/41 (90.2)	33/41 (80.5)	28/41 (68.3)
mCB		39/41 (95.1)	37/41 (90.2)	34/41 (82.9)
Salmonella	40[a]			
Feces		40/40 (100)	35/35 (100.0)	40/40 (100)
FSTM		40/40 (100)	35/35 (100.0)	40/40 (100)
mCB		40/40 (100)	35/35 (100.0)	39/40 (97.5)
STEC	23[a]			
Feces		23/23 (100)	22/22 (100)	23/23 (100)
FSTM		23/23 (100)	21/22 (95.5)	23/23 (100)
mCB		23/23 (100)	22/22 (100)	23/23 (100)
Shigella	14			
Feces		13/14 (92.9)	11/14 (78.6)	11/14 (78.6)
FSTM		13/14 (92.9)	11/14 (78.6)	11/14 (78.6)
mCB		13/14 (92.9)	13/14 (92.9)	12/14 (85.7)
Yersinia	16[a]			
Feces		16/16 (100)	14/15 (93.3)	13/16 (81.3)
FSTM		16/16 (100)	15/15 (100)	14/16 (87.5)
mCB		16/16 (100)	15/15 (100)	15/16 (93.8)

[a] At the 4-day time point, viability assessment of 5 *Salmonella*, 1 STEC, and 1 *Yersinia* specimen(s) was missed and therefore not included in the denominator for the percent viability calculation.

specimens that did not grow at the time of culture for the study had been collected 4 and 7 days prior to time zero for the 7-day viability assessment. Of the 3 *Shigella* specimens that did not grow culture for the study, the time from collection is known for only 1 specimen (11 days).

4. Discussion

Previously published studies have compared the effect of different transport media on improving the recovery of enteric bacteria by culture, but clinical fecal specimens in the absence of transport media were not compared using the same specimens (Wang et al., 1983; Cary and Blair 1964; Wasfy et al., 1995; Mundy et al., 1991). Given that these organisms are routinely isolated from feces, it is a reasonable assumption that fecal material could be the most appropriate transport medium for preserving the viability of these organisms. Our study demonstrates that fecal material itself is an adequate transport medium for some organisms (STEC and *Salmonella*) and not others such as *Campylobacter*.

Our results also demonstrate that transport media are not needed for the recovery of STEC and *Salmonella*. All STEC-positive stool specimens sent to ProvLab for inclusion in the study grew upon receipt despite the lack of transport media and a median of 7 days and range of 1–106 days from collection to time of culture for the study (day 0). Furthermore, no loss of viability was observed in any specimen in the 7-day storage experiment with or without transport media. *Salmonella* also demonstrated a very high recovery rate of >95% at time of receipt at ProvLab with days from collection ranging from 3 to 33 days and 100% recovery in the 7-day experiment w/oTM.

Based on our findings, the role of transport media in improving the recovery of *Shigella* and *Yersinia* is also questionable if the turnaround time to culture is less than 2 or 4 days, respectively. For *Shigella*, more than 93% of fecal specimens had organism recovered after 2 days regardless of the storage condition. For *Yersinia,* all fecal specimens had recoverable organism from feces without transport media at 2 days and ~93% at 4 days. mCB did appear to offer some improvement in organism viability for *Shigella* at 4 days and *Yersinia* at 7 days, but to make this conclusion, we would need more specimens, which were not available due to the low prevalence of these organisms in our population.

Our findings do however support recommendations that mCB be used as a transport media to enhance the recovery of *Campylobacter* (Baron 2011; Humphries and Linscott 2015; Pillai 2016). Unlike *Salmonella,* STEC, or *Yersinia, Campylobacter* could not be recovered from the day 2 time point from all stools, and organism was only recovered in 71% of feces stored w/oTM and 68% stored in FSTM after 7 days at 4 °C. When feces were stored with mCB, organism could be recovered from 10% more specimens than w/oTM or FSTM. Despite these findings, if the turnaround time for culture was less than 2 days, the addition of transport media to the fecal specimen may not be of significant clinical benefit because the recovery was found to be >90% at 2 days of storage w/oTM.

The ability of FSTM to preserve viability has previously been evaluated by comparing to other transport media not designed for stool such as Stuart's or the E-Swab™ Medium by Copan (Hirvonen and Kaukoranta 2014). The findings herein demonstrate that storage in FSTM did not appear to confer any advantage over feces w/oTM, exemplifying the importance to test new transport media against the standard (mCB) and feces w/oTM. It is important to note that CLSI M40-A2 (CLSI 2014) recommends using planktonic bacteria for the evaluation of transport media and not specimen. This approach may be appropriate for quality control but not for evaluating the effectiveness of a transport media. Validation of a transport media should represent the real world and thus should be done by using specimens tested and collected for clinical diagnosis.

One limitation of our study design was that not all feces were obtained fresh and, therefore, not all feces had viable organism upon culture at ProvLab. As a result of this limitation, we were able to collect additional valuable data on the recovery of the pathogens in fecal specimens stored w/oTM, but there is also the possibility that we have selected for the inclusion in our 7-day viability study fecal specimens with higher bacterial load or strains with better survival characteristics. We did not find evidence for this concern to be true. This was not an

Table 3
Viability of organisms in feces at time of receipt ($T = 0$ day) for all specimens and for those specimens with date of collection available (with date ranges for each organism).[a,b]

	All specimens received		Specimens with collection time available				
Organism	No. fecal specimens received	No. viable at $T = 0$ h (%)	No. fecal specimens	No. viable at $T = 0$ h (%)	Avg. collection to $T = 0$ h	Median collection to $T = 0$ h	Range
Campylobacter	66	42 (63.6)	66	42 (63.6)	10	6	3 to 43
Salmonella	48	46 (95.8)	46	44 (95.6)	11	9	3 to 33
STEC	24	24 (100)	22	22 (100)	15	7	1 to 106[a]
Shigella	19	16 (84.2)	16	15 (93.8)	14	7	3 to 86[a]
Yersinia	21	17 (80.9)	19	15 (78.9)	7	6	3 to 21

[a] One specimen of STEC was received after 106 days and grew at $T = 0$ day; the other specimens ranged from 1 to 29 days.
[b] One specimen of *Shigella* was received after 86 days and grew at $T = 0$ day; the other specimens ranged from 3 to 25 days.

issue for STEC or *Salmonella* because all specimens were viable at time of culture for the study for STEC and >95% for *Salmonella*. Nor was this an issue for *Campylobacter* as the time from collection to culture for the study was similar for those samples that were culture positive and negative. Specimen numbers for *Yersinia* and *Shigella* were too small to make an assessment in this regard. Performing this experiment in a real-world setting by placing feces in a container alone and in transport media at collection time (or even receipt by the front-line laboratory) would be ideal.

In conclusion, laboratories not using mCB should re-consider if fecal transport media are required. Such a decision should be made by taking into consideration the time from collection to culture and, in the context of CIDT, which organisms need to be recovered. Novel fecal transport media should be evaluated by comparing to fecal specimens w/oTM and mCB with an adequate number of clinical feces. Although this is a pilot study, it still provides valuable insight on the viabilities of different enteric bacteria in feces versus transport media, which is a crucial factor in the era of CIDT.

Author statements

Author contributions

BMB and LC contributed to conceptualization, methodology, and supervision. BMB performed formal analysis. CF contributed to methodology and investigation. All authors contributed to all stages of manuscript preparation.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

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