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Note

Enhanced culture recovery of *Campylobacter* with modified Cary-Blair medium: A practical field experience

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ABSTRACT

Modified Cary-Blair medium derived devices have been implemented in many laboratories to optimize culture recovery of common bacterial enteric pathogens. Our analysis constitutes the first report of routine laboratory experience supporting the idea that the use of such devices enhances *Campylobacter* recovery from stools.

Campylobacter commonly causes gastroenteritis worldwide. As *Campylobacter* are fastidious bacteria, more and more laboratories are adopting specific antigen tests (Fitzgerald et al., 2016) or molecular tests (Zhang et al., 2015) for their detection in stools. Since increasing antimicrobial resistances are reported in *Campylobacter* (Ge et al., 2013), strain isolation remains essential for antimicrobial susceptibility testing and epidemiologic purposes (Couturier, 2016). The modified Cary-Blair (MCB) medium for transport and preservation was recommended to optimize culture recovery of common bacterial enteric pathogens (*Campylobacter, Salmonella* and *Shigella*) in stools (Wang et al., 1983). However, the performance of MCB medium has never been reported in routine clinical laboratory conditions.

In the bacteriology laboratory of the Toulouse University Hospital (France), we retrospectively evaluated the impact of implementation of the MCB medium FecalSwab (Copan, Brescia, Italy) on *Campylobacter, Salmonella* and *Shigella* isolation rate from clinical stools. Before 2013, all fecal specimens were collected in dry containers. Since 2013, stools from the Children's Hospital have been collected in FecalSwab devices *i.e.* a tube with 2 mL of MCB medium and a flocked swab, whereas adult specimens were still transported in dry containers. To assess the impact of these changes, we compared the distributions of positive samples for each common enteric bacterial pathogen (*i.e. Salmonella, Shigella,* and *Campylobacter*) in 2012 and 2014 for children (*i.e.* patients under the age of 15) and adults using the Chi-square test (statistical significance was defined as a p-value $< .05$). Because MCB medium was implemented in mid-2013, we chose to exclude 2013 from our analysis to avoid any bias due to seasonal variation in common enteric bacterial infections.

During the studied periods, fecal samples were inoculated within 24 h on Campylosel agar plates (bioMérieux, Marcy-l'Etoile, France) incubated in a micro-aerobic atmosphere at 35 ± 2 °C for 48 h, on Rambach agar plates (VWR International, Radnor, Pennsylvania, USA), Bromocresol Purple agar plates (bioMérieux) and in Selenite broth (Copan) incubated in an aerobic atmosphere at 35 ± 2 °C for 24 h. The Selenite broth was then subcultured onto a Rambach agar plate incubated in an aerobic atmosphere at 35 ± 2 °C for 24 h. All suspected colonies were identified by biochemical analyses (API Campy, bioMérieux, for Campylobacter isolates or GN cards on Vitek 2 system bioMérieux, for *Salmonella* and *Shigella*) during both periods.

The number of specimens and patient characteristics (number, age) were comparable in 2012 and 2014 (Table 1). Distribution of pediatric samples positive for *Campylobacter* was significantly different between 2012 and 2014 (2.76%, $n = 50$ *vs.* 4.35%, $n = 71$; $p = .011$) whereas no statistical difference was observed for adults (1.33%, $n = 59$ *vs.* 1.62%, $n = 65$; Table 1). In comparison, the distributions of samples positive for *Salmonella* or *Shigella* were not statistically different between 2012 and 2014 for both children and adults. The distribution of *Campylobacter* species was similar between 2012 and 2014 for both pediatric and adult samples, *C. jejuni* being the most frequently isolated species.

The specific increase in *Campylobacter* recovery in pediatric stools between 2012 and 2014 suggests that collection in FecalSwab improves *Campylobacter* preservation and detection. Indeed, no such trend was observed in adult samples for which dry containers were used for both

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Table 1
Characteristics of pediatric and adult stool samples collected in 2012 and 2014.

	Pediatric stool samples		Adult stool samples	
	2012	2014	2012	2014
	Dry containers	MCB medium	Dry containers	Dry containers
Patients				
Number of patients	1319	1250	2895	2612
Mean Age (years)	1.88	2.08	61.81	60.46
Standard deviation for age (years)	3.27	3.49	20.20	20.34
Number of samples	1812	1632	4426	4004
Positive samples				
Campylobacter	50 (2.76%)	71*(4.35%)	59 (1.33%)	65 ns (1.62%)
Among which				
C. jejuni	45	59 ns	43	51 ns
C. coli	4	10 ns	9	7 ns
C. fetus	–	–	3	3
C. hyointestinalis	–	–	–	1
C. upsaliensis	–	–	1	–
Campylobacter sp.	1	2	3	3
Salmonella	57 (3.15%)	58 ns (3.55%)	24 (0.54%)	34 ns (0.85%)
Shigella	6 (0.33%)	10 ns (0.61%)	7 (0.16%)	9 ns (0.22%)

nsNo statistical difference between 2012 and 2014 according to Chi-square test.
* Statistical difference ($p < .05$).

periods. Of note, no specific *Campylobacter, Salmonella* or *Shigella* outbreak was reported during the study. No increase in the incidence of *Campylobacter* infections was reported by the French National Reference Centre for *Campylobacter* between 2012 and 2014, although the number of reported cases increased because of the notification system improvement (King et al., 2012; Van Cauteren et al., 2014). In our study, MCB medium implementation led to a global increase in *Campylobacter* recovery, but it did not enhance the recovery of any specific *Campylobacter* species. This result should be interpreted with caution because of the small numbers of non-*jejuni Campylobacter*-positive samples.

The implementation of MCB medium for preservation of pediatric stools up to 24 h did not lead to an increase in *Salmonella* or *Shigella* recovery, as expected according to the literature (Altieri et al., 2011). The specific impact on *Campylobacter* detection might be due to its particular fragility compared to other enteric bacterial pathogens.

MCB medium was shown to be suited for *Campylobacter* preservation in studies using turkey cecal specimens (Luechtefeld et al., 1981) and for collection of human stools kept at 4 °C prior to evaluation (Wang et al., 1983). Inoculated with ATCC strains, FecalSwab demonstrated better preserving properties than Amies-based medium which is not an MCB derived medium (Hirvonen and Kaukoranta, 2014). In a study including 125 stools analyzed in accordance with current laboratory procedures, FecalSwab was superior to dry container as a transport device, recovering 10 *vs.* 7 *Campylobacter jejuni* strains (Altieri et al., 2011). Conversely to these early side-by-side studies performed on small cohorts and artificially inoculated stools, our analysis was conducted on several thousand samples over 2 years processed with routine laboratory procedures. Our work constitutes the first report of routine laboratory experience supporting the idea that the implementation of MCB derived devices enhances *Campylobacter* recovery from stools after preservation at room temperature for < 24 h.

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The authors declare no conflict of interest.

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