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CASA Chromogenic Medium for Enteric *Campylobacter* Species[▽]

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We prospectively assessed stool samples from 370 patients for *Campylobacter* species by comparing three selective agar media incubated at two temperatures: 42°C and 37°C. Twenty patients (5.4%) were found positive. The chromogenic medium CASA (AES Chemunex, France) proved highly efficient for *C. jejuni* and *C. coli* recovery, while lessening the workload in the lab.

Campylobacter species, especially the thermophilic species *C. jejuni* and *C. coli*, are known worldwide to be the most common causal agents (1, 2, 4) of bacterial gastroenteritis. Culture of the responsible strain is of importance for confirmation of *Campylobacter* infection (3) because extraintestinal complications (e.g., bacteremia, meningitis, arthritis, cholecystitis, and Guillain-Barré syndrome) may occur (7, 12). It is therefore of concern that *Campylobacter* detection in stools by culture methods may lack sensitivity (4, 9, 12). Furthermore, acquired resistance to antibiotics is increasing, and the isolation of the strain responsible for an infection is necessary for *in vitro* antimicrobial susceptibility and resistance evaluation (8, 10). Because of the abundance of competitive flora, selective media are necessary for the recovery of *Campylobacter* from stool samples. Alternatively, nonselective media (chocolate agar plus PolyViteX [PVX], for instance) in association with a 0.65-μm-pore-size membrane filter, which *Campylobacter* can cross, have been proposed (13). This procedure is not in general use in clinical laboratories and was not utilized in this study. In the present study we compared the newly introduced *Campylobacter* selective chromogenic medium CASA (AES Chemunex) with two routinely employed selective agar media, Karmali (AES Chemunex) (5) and Campylosel (bio-Mérieux). We studied their ability to support the growth of various *Campylobacter* species and their performance in thermophilic *Campylobacter* detection from stools in real-world clinical settings.

(Some of these data were presented in part as a poster at the 110th General Meeting of the American Society for Microbiology, San Diego, CA, 23 to 27 May 2010 [6a].)

Fresh cultures of 8 different species belonging to the genus *Campylobacter* were suspended in Mueller-Hinton broth to reach a McFarland turbidity of 1. One microliter of a 1 to 1,000 dilution of these suspensions was plated onto the selective media CASA, Karmali, and Campylosel and the nonselective medium chocolate agar plus PVX as a growth control and incubated for 96 h at both 42°C and 37°C under a microaerobic atmosphere (device BACT-R [Sobioda, Montbonnot-Saint-Martin, France] and corresponding jars). *C. jejuni*, *C. coli*, *C. lari*, and *C. fetus* subsp. *fetus* exhibited comparable levels of growth on all 4 media. The growth of *C. hyointestinalis* on CASA, Karmali, and Campylosel media corresponded to 50% of the growth observed on chocolate agar plus PVX. *C. upsaliensis*, *C. sputorum* subsp. *bubulus*, and *C. fetus* subsp. *venerealis* showed growth only on chocolate agar plus PVX. Unlike *C. sputorum* subsp. *bubulus* and *C. fetus* subsp. *venerealis*, *C. hyointestinalis* and *C. upsaliensis* are agents of human gastroenteritis (11). The use of selective media has already been shown to lack sensitivity for the detection of the latter two species (6).

Fresh cultures of 79 different microbial strains belonging to 24 species commonly found in stools were suspended in Mueller-Hinton broth to reach a McFarland turbidity of 1 and were then diluted in sterile saline water at ratios of 1 to 1,000 (high inoculum) and 1 to 100,000 (low inoculum). Ten microliters of each of these suspensions was plated onto the selective CASA, Karmali, and Campylosel media and the nonselective tryptic soy agar as a growth control and incubated for 96 h at 37°C under a microaerobic atmosphere. All *Campylobacter* strains grew well on the tryptic soy agar plates. No growth for the following bacteria was observed on the CASA, Karmali, and Campylosel media: *Enterobacter cloacae* ($n = 3$), *Enterobacter aerogenes* ($n = 2$), *Klebsiella terrigena* ($n = 1$), *Serratia marcescens* ($n = 2$), *Klebsiella pneumoniae* ($n = 5$), *Klebsiella oxytoca* ($n = 5$), *Proteus mirabilis* ($n = 3$), *Shigella sonnei* ($n = 2$), *Salmonella enterica* serovar Typhimurium ($n = 5$), *Citrobacter freundii* ($n = 2$), *Aeromonas hydrophila* ($n = 2$), *Yersinia enterocolitica* ($n = 2$), *Staphylococcus aureus* (methicillin resistant, $n = 3$; methicillin susceptible, $n = 3$), *Streptococcus agalactiae* ($n = 2$), *Pseudomonas putida* ($n = 2$), and *Pseudomonas fluorescens* ($n = 2$). Some enterobacteriaceae exhibited growth only on Karmali medium (Table 1). Growth of enterococci on Campylosel and Karmali was observed. There was only weak growth of *Enterococcus faecium* on the CASA medium. One of the five strains of *Pseudomonas aeruginosa* gave tiny colonies on both Karmali and Campylosel media. Growth of *Candida albicans* was inhibited on Campylosel medium, but the yeast grew on Karmali and CASA media. On CASA medium, *Candida albicans* first grew as white colonies and became pink after 48 to 72 h.

From May 2009 to October 2009, 370 diarrheic stool samples, mainly from pediatric out- and inpatients (within the first 5 days after admission to the local public teaching hospital), were prospectively analyzed. Samples were inoculated onto CASA, Karmali, and Campylosel and incubated at both 37°C and 42°C under a microaerobic atmosphere. Cultures were observed after 24, 48, 72, and 96 h and checked for "*Campy-*

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TABLE 1. Selective growth of different intestinal bacteria and yeast on various media

Species	n[a]	Growth[b] (H/L[c]) at 37°C on:		
		CASA	Karmali	Campylosel
Escherichia coli	9	0/0	0*/0*	0/0
Enterobacter gergoviae	1	0/0	+/0	0/0
Pseudomonas aeruginosa	5	0/0	0*/0*	0*/0*
Providencia stuartii	2	0/0	+/+	0/0
Morganella morganii	2	0/0	++/+	0/0
S. enterica serovar Hadar	1	0/0	+/+	0/0
S. enterica serovar Heidelberg	1	0/0	+/0	0/0
Enterococcus faecalis	3	0/0	0*/0*	0*/0*
E. faecium (vancomycin susceptible)	5	+/0	++/++	++/++
E. faecium (vancomycin resistant)	1	+/+	++/++	++/++
C. albicans	3	++/++	++/++	0/0

[a] *n*, number of strains.
[b] + and ++, growth corresponding to ≤50% and 100% of the growth on tryptic soy agar, respectively; 0, no growth; *, growth of 1 strain was +/+ or ++/+.
[c] H/L, high/low inoculum (see text).

lobacter-like" colonies (Campylosel and Karmali) and red colonies (CASA). Suspect colonies were subsequently studied by wet mount, Gram stain, by oxidase test, and by determination of growth characteristics (temperature, microaerophily), hippurate levels, and indoxyl acetate hydrolysis and were identified by Api Campy (bioMérieux) if required. Because falsely positive results from culture were improbable, we considered the isolation of *Campylobacter* from any medium at any temperature as the gold standard. Among the 370 analyzed stool samples, 20 (5.4%) were found positive for *C. jejuni* ($n = 17$) or *C. coli* ($n = 3$) for patients with ages from 1 month to 25 years (median = 6.5 years). All three *C. coli* isolates were detected with the three media incubated at both 37°C and 42°C. Among the 17 samples positive for *C. jejuni*, the strains were recovered 17 times on CASA medium (16 at 37°C and 16 at 42°C), 16 times on Campylosel medium (14 at 37°C and 16 at 42°C), and 15 times on Karmali medium (14 at 37°C and 15 at 42°C) (Table 2).

The detection of *Campylobacter* colonies on culture media is a crucial step in the microbiological diagnosis of *Campylobacter*-induced diarrhea. On the routinely employed selective *Campylobacter* media, it may be difficult to pick out *Campylobacter* colonies that are difficult to differentiate among a polymorphic flora, particularly when the inoculum is low. On the selective chromogenic CASA agar, there is a strong inhibition of growth of the competitive bacteria from the intestinal flora and *Campylobacter* colonies appear red and are easily detected. In 260 samples, suspect colonies (1 to 3 morphology types) that were not identified as *Campylobacter* had to be worked up at least by wet mounting in *Brucella* broth followed by Gram staining, subculturing, and verification of the microaerophilic character. Suspect colonies from all these 260 samples appeared on either Karmali or Campylosel or both media, whereas red non-*Campylobacter* colonies were present on CASA agar in only 24 of the 260 samples; these appeared after 48 to 72 h of incubation and were either nonfermenting Gram-negative rods (2 samples) or yeast (22 samples) (Table 3). There was a slight selective advantage to incubating CASA at 42°C rather than at 37°C (Tables 2 and 3). For the remaining 90 samples, no colonies grew on any media at either incubation temperature. The use of CASA medium was therefore associated with a decrease in unnecessary confirmation tests. We calculated that the times required to check non-*Campylobacter* colonies by microscopic analysis of bacterial motility in *Brucella* broth were 1.5, 3, and 4.5 min for 1 colony, 2 colonies, and 3 colonies, respectively. Consequently, the times needed for the analysis of non-*Campylobacter* colonies were estimated to be 3.5 and 1.4 h per 100 stool samples with Karmali and Campylosel, respectively, while it was only 0.2 h per 100 stool samples with CASA (Table 3). Moreover, the number of positive *Campylobacter* culture stool samples was equal to or slightly better with CASA medium than with both Karmali and Campylosel media at both 42°C and 37°C, demonstrating that there was no loss of sensitivity with the use of CASA media (Table 2).

Our results clearly show that the CASA medium is highly selective against most of the culturable species of the intestinal flora without any loss of sensitivity for the diagnosis of *C. jejuni*- and *C. coli*-induced diarrhea. This chromogenic agar contributes significantly to reducing the workload in the clinical microbiology laboratory.

TABLE 2. Sensitivity and negative predictive values of CASA, Campylosel, and Karmali media for the detection of *Campylobacter*

Inc[a] temp (°C)	Medium	No. of stool samples positive/negative for *Campylobacter*[b]	Sen[c] (%)	NPV[d] (%)
37	CASA	19/1	95	99.7
	Campylosel	17/3	85	99.1
	Karmali	17/3	85	99.1
42	CASA	19/1	95	99.7
	Campylosel	19/1	95	99.7
	Karmali	18/2	90	99.4

[a] Inc, incubation.
[b] Twenty of 370 samples were positive, as defined in Results, for *C. jejuni* or *C. coli*.
[c] Sen, sensitivity.
[d] NPV, negative predictive value.

TABLE 3. Time spent to analyze non-*Campylobacter* colonies

Inc temp (°C)	Medium	No. of samples with indicated no. of non-*Campylobacter* colony morphology types				Time NCA[a] (h/100 stool samples)
		0	1	2	3	
37	CASA	236	24	0	0	0.23
	Campylosel	131	64	55	10	1.96
	Karmali	0	84	143	33	4.51
42	CASA	238	22	0	0	0.21
	Campylosel	157	64	34	5	1.41
	Karmali	12	138	99	11	3.55

[a] Time NCA, time spent for the analysis of non-*Campylobacter* colonies.

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