

CASA CHROMOGENIC MEDIUM FOR ENTERIC CAMPYLOBACTERS

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Abstract

Among bacterial agents responsible for infectious diarrhea, those belonging to *Campylobacter* genus, especially thermophilic species *C. jejuni* and *C. coli*, are worldwide found to be the first causative agents, moreover when pediatric patients are considered.

We have compared routine detection of *campylobacters* from 370 stool samples from mainly pediatric out and in-patients (samples within the 5 first days after admittance). Samples were seeded in parallel onto six plates : two selective agars of general use, (Karmali and Campyloset[®]) and the chromogenic agar CASA[®] incubated at both 37°C and 42°C under microaerophilic condition. Cultures were observed after 24, 48, 72 and 96h of incubation and checked for « *campylobacter-like* » colonies (Campyloset and Karmali) and red colonies (CASA).

Twenty (5.4%) patients, aged from one month to 25 years (median = 6.5 years), were found positive for *C. jejuni* (N =17) or *C. coli* (N = 3).

Concerning the three *C. coli* positive samples, all media incubated at both temperatures allowed the recovery of the strain.

Among the 17 samples positive for *C. jejuni*, the strain could be recovered from :

CASA: 17 times (16 at 37°C and 16 at 42°C)

Campyloset: 16 times (14 at 37°C and 16 at 42°C)

Karmali: 15 times (14 at 37°C and 15 at 42°C)

No colony grew on any media at any incubation temperature in 90 cases. Suspect colonies had to be worked up from either Karmali or Campyloset or both for all 260 remaining samples while red non-*campylobacter* colonies were present on CASA agar in 24 cases only. Those last ones appeared after 48 to 72h incubation and were either non fermenting Gram-negative rods (twice) or yeasts (22 times).

Thermophilic *campylobacters* detections might be really tedious because of the needs for multiply colonies repeated checking (shape and motility at least). The use of the chromogenic medium both gave a strong inhibition of competitive flora and allowed an easy screening of red typical *campylobacter* colonies corresponding to a better productivity by lessening the workload in the lab without loss of sensitivity.

Background

Among bacterial agents responsible for infectious *diarrhoea*, those belonging to *Campylobacter* genus, especially thermophilic species *C. jejuni* and *C. coli*, are worldwide found to be the first causative agents, moreover when pediatric patients are considered. As the current case definition for *Campylobacter* requires culture confirmation and because extra-intestinal disease may complicate the initial episode, culture of the responsible strain is of importance especially for susceptibilities when needed as acquired resistances to antimicrobial are increasing by those bacteria also.

A whole range of factors may influence the results of *campylobacter* cultures, i.e., the transport time, the different types of selective agar media used, the number of media used, the incubation time, use of correct temperature and incubation atmosphere...

Objective

The present study is an evaluation of the real-world performance characteristics of a newly introduced *campylobacter* selective chromogenic medium CASA (AES Chemunex) in comparison to two selective agars of general use: Karmali (AES Chemunex) and Campyloset[®] (bioMérieux) for isolation of thermophilic *campylobacters* from stools.

Methods

370 stool samples were collected from out and in-patients (within the 5 first days after admittance at town public teaching hospital) with diarrhoea.

Samples were seeded in parallel onto six plates : two selective agars of general use, (Karmali and Campyloset[®]) and the chromogenic agar CASA[®] incubated at both 37°C and 42°C under microaerophilic condition (device BACT-R[®], Sobioda, Montbonnot-Saint-Martin, France and corresponding jars). Cultures were observed after 24, 48, 72 and 96h of incubation and checked for « *campylobacter-like* » colonies (Campyloset and Karmali) and red colonies (CASA).

Suspect colonies were subsequently identified by wet mount, Gram stain, oxidase, growth conditions (temperature, microaerophily), hippurate and indoxyl acetate hydrolysis plus Api campy (bioMérieux).

Results

Among the 370 stool samples, 20 (5.4%) were found positive for *C. jejuni* (N =17) or *C. coli* (N = 3) for patients aged from one month to 25 years (median = 6.5 years) (Table 1).

Table 1 : stool positive for *campylobacters* (n = 20)

(a): species ; (b): N = not recovered

Patient age	CASA 42°C	Karmali 42°C	Campyloset 42°C	CASA 37°C	Karmali 37°C	Campyloset 37°C
1mo	<i>C. jejuni</i> (a)	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>
10y - 2mo	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	N(b)	<i>C. jejuni</i>	N
5y - 6mo	<i>C. jejuni</i>	N	<i>C. jejuni</i>	<i>C. jejuni</i>	N	<i>C. jejuni</i>
1y - 3mo	<i>C. jejuni</i>	<i>C. jejuni</i>	N	<i>C. jejuni</i>	<i>C. jejuni</i>	N
10y - 2mo	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>
15y - 9mo	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>
14y - 3mo	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>
5y	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	N	N
7y - 11mo	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>
1y	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>
4mo	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>
10mo	N	N	<i>C. jejuni</i>	<i>C. jejuni</i>	N	<i>C. jejuni</i>
5y - 2mo	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>
6y - 2mo	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>
8y - 6mo	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>
11y - 4mo	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>
6y - 8mo	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>	<i>C. jejuni</i>
3y - 10mo	<i>C. coli</i>	<i>C. coli</i>	<i>C. coli</i>	<i>C. coli</i>	<i>C. coli</i>	<i>C. coli</i>
3mo	<i>C. coli</i>	<i>C. coli</i>	<i>C. coli</i>	<i>C. coli</i>	<i>C. coli</i>	<i>C. coli</i>
25y - 8mo	<i>C. coli</i>	<i>C. coli</i>	<i>C. coli</i>	<i>C. coli</i>	<i>C. coli</i>	<i>C. coli</i>
Total	19	18	19	19	17	17

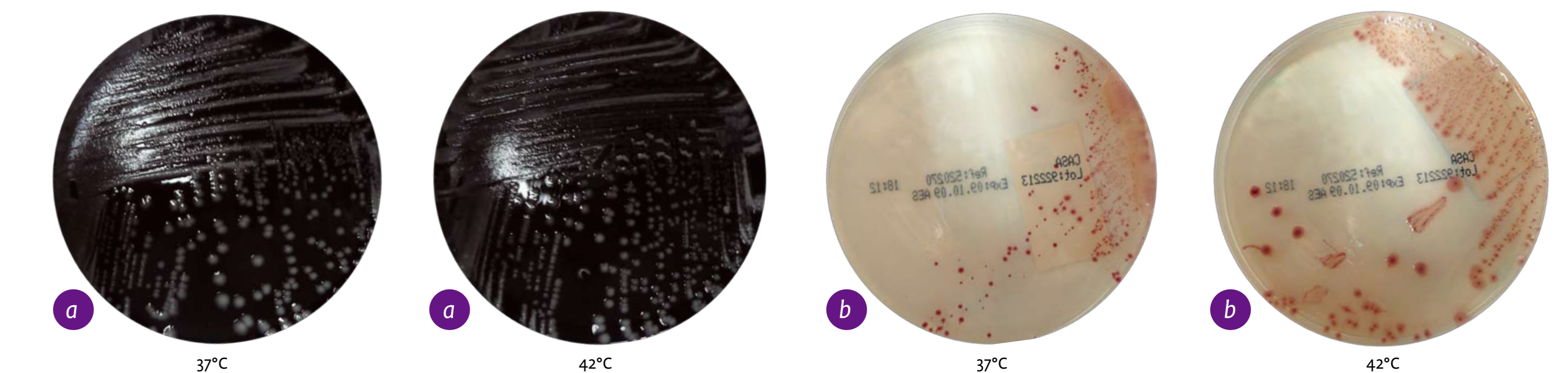
No colony grew on any media at any incubation temperature for 90 samples.

Suspect colonies (1 to 3 morphology types) which eventually were not *campylobacters* had to be worked up (wet mount and Gram stain, subcultures and respiratory type if needed) from either Karmali or Campyloset or both for all the 260 remaining samples. Red non-*campylobacter* colonies were present on CASA agar in 24 cases : those appeared after 48 to 72h incubation and were either non fermenting Gram-negative rods (2 times) or yeasts (22 times) (Table 2).

Table 2 : numbers of types of suspect colonies that were eventually non-*campylobacter* from each media for 260 stool samples

Numbers of colonies types	Numbers of stool samples with those colonies and media concerned:					
	CASA 42°C	Karmali 42°C	Campyloset 42°C	CASA 37°C	Karmali 37°C	Campyloset 37°C
0	237	12	157	236	0	131
1	23	138	64	24	84	64
2	0	99	34	0	143	55
3	0	11	5	0	33	10
Total samples	260	260	260	260	260	260

Figure 1: same stool sample plated onto traditional Karmali (a) and chromogenic CASA[®] (b) media; red colonies on CASA[®] were *campylobacters* whereas no *campylobacter* colony could eventually be recovered, amidst the competitive flora, from Karmali agar at both temperature 37°C or 42°C



Discussion

Reliable diagnosis of digestive tract infections due to *campylobacter* is important and accurate cultures are necessary: while the majority of cases of *campylobacter* diarrhoea may be mild and self limiting, severe illness with a non specific presentation may occur especially by the elderly, infants, or immuno-compromised patients. Isolation of the responsible strain is needed for susceptibilities which may be necessary, as *campylobacter* will show acquired resistances towards some antibiotics regimens currently used, fluoro-quinolones especially. One may therefore be concerned about the possible insensitivity of *campylobacter* cultures. This may be of concern too for Public Health people as current CSTE and FoodNet *Campylobacter* case definitions require culture confirmation.

Overgrowth by normal flora on the regular *campylobacter* media is a problem especially when culture densities make it difficult to pick out a few *campylobacter* colonies that poorly differentiate among some non-*campylobacter* but *campylobacter*-mimicking colonies. The location of colonies on culture media is a crucial step in the microbiological diagnosis of *Campylobacter's diarrhoeas*. Morphologies and characteristics of these colonies are quite uneasily differentiated from some varied other non *campylobacter* colonies which therefore have to be worked-up. On chromogenic agar CASA[®], *campylobacter* appear as red colonies which facilitate the detection compared to both Karmali or Campyloset[®]. The use of CASA[®] was therefore associated with a decrease of unnecessary confirmation tests (Table 2). Also CASA[®] medium showed a strong selectivity. Despite that selectivity, the number of *Campylobacter* culture positive stools was equal or even slightly better (Table 1) with CASA[®] when compared to both Karmali and Campyloset[®] at both 42°C and 37°C. This may be both due to very specific selectivity and easier detection of *Campylobacter* colonies on CASA[®]. According to these results, we conclude that the new chromogenic agar CASA[®] supplied by AES CHEMUNEX is efficient for *Campylobacter* isolation from stool samples and contributes to reduce the workload in the clinical microbiology laboratory.

References

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