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Comparison of CHROMagar Yersinia and CIN Agar media for isolation of potentially virulent *Yersinia enterocolitica* in stools

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Background

Yersinia enterocolitica isolates are responsible for numerous intestinal infections in humans. Although widely used for isolation of *Y. enterocolitica* in stools, cefsulodin-irgasan-novobiocin (CIN) agar lacks the ability to differentiate potentially virulent and non-pathogenic isolates. CHROMagar Yersinia (CAY) is a new chromogenic medium recently developed for isolation and presumptive identification of enterovirulent *Y. enterocolitica* biotypes as mauve colonies after 48 h (FIG.1).

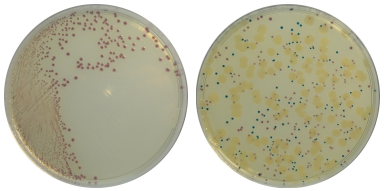

FIG. 1. Colonies of pathogenic *Y. enterocolitica* on CHROMagar Yersinia after 48 h at 30°C. Left: pure growth of characteristic mauve colonies. Right: mixed culture with *Providencia rettgeri* (beige colonies) and *Citrobacter freundii* (blue-green colonies), two frequently encountered species yielding false-positives when grown on CIN agar

Materials & Methods

- We first evaluated the growth and colonial aspect on CAY of 90 stock strains of 9 *Yersinia* species, including 40 virulent and 9 avirulent *Y. enterocolitica* isolates.
The presence of the pYV virulence plasmid characteristic of pathogenic strains was assessed by PCR of pYV-localized gene *yadA* in all *Y. enterocolitica* isolates (TABLE 1).
All *Y. enterocolitica* isolates were tested for esculin hydrolysis and serotyped (SIFIN, Germany) (TABLE 1).

TABLE 1. Serotypes and virulence marker of the 49 stock isolates of *Y. enterocolitica* studied

Serotype	O3	O5		O8	O9	O27	O16-29	non typable
pYV status (no. of strains)	pYV$^+$ (12)	pYV$^+$ (8)	pYV$^-$ (1)	pYV$^+$ (8)	pYV$^+$ (5)	pYV$^+$ (6)	pYV$^+$ (1)	pYV$^-$ (8)

- CAY and CIN agar were then prospectively compared for the detection and presumptive identification of pathogenic *Y. enterocolitica* on 1,500 consecutive diarrheic stool specimens.
Bacterial identifications were obtained on a Microflex MALDI-TOF mass spectrometer (Brüker, France), and 16S rDNA sequencing was performed when required.

Results

Growth of *Yersinia* spp. stock isolates on CHROMagar Yersinia (TABLE 2). All pathogenic (*yadA*$^+$, esculin$^-$) *Y. enterocolitica* yielded typical mauve colonies on CAY (FIG. 2A) as did the 3 *Yersinia bercovieri* isolates tested. All non-pathogenic *Y. enterocolitica* (*yadA*$^-$, esculin$^+$) grew as blue-green colonies (FIG. 2B). None of the 19 *Y. pseudotuberculosis* isolates tested grew on CAY.

TABLE 2. Aspect of stock isolates of 9 *Yersinia* species on CHROMagar Yersinia after 48 h at 30°C.

Species (no. of isolates)	Colonial morphology	
	Color	Size
Y. enterocolitica (49)		
virulent/pYV$^+$ (40)	mauve	1-2 mm
avirulent/pYV$^-$ (9)	blue metal	1-3 mm
Y. aldovae (4)	white	0.5 mm
Y. bercovieri (3)	mauve	1-2 mm
Y. frederiksenii (3)	blue metal	0.5-1 mm
Y. intermedia (2)	blue metal	0.5-1 mm
Y. kristensenii (5)	mauve streaks	< 0.5 mm
Y. mollaretii (3)	mauve	0.5 mm
Y. pseudotuberculosis (19)	no growth	
Y. ruckeri (2)	no growth	

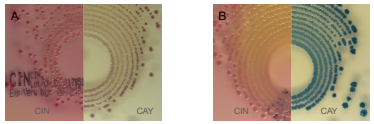

FIG. 2. Colonial aspect of pathogenic and non-pathogenic *Y. enterocolitica* on CIN and CHROMagar Yersinia (CAY) after 48h at 30°C.
(A) Pure growth of a pathogenic O3 isolate. (B) Non-pathogenic O5 isolate.

Prospective stool analysis.

- Six pathogenic *Y. enterocolitica* isolates were obtained from the 1,500 stool samples (0.4 %). All isolates were recovered from both CIN and CAY media (sensitivity, 100%).

- False positive on CAY were *Stenotrophomonas maltophilia* (n = 9) (Fig. 3A), *Shewanella putrefaciens* (n = 1) (Fig. 3B) , *Yersinia bercovieri* (n = 1) and *Brevundimonas terrae* (n = 3), a species previously unknown in this setting.

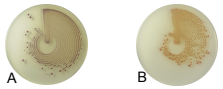

FIG. 3. Stool-derived false positives on CHROMagar Yersinia.
(A). *Stenotrophomonas maltophilia*. (B). *Shewanella putrefaciens*. Incubation 48 h at 30°C.

- False positives on CIN (red "bullseye" colonies) were mainly *Citrobacter freundii* (n = 88, FIG.4) and *Providencia* spp. (n = 35), *Aeromonas* spp. (n = 12), and non-pathogenic *Y. enterocolitica* biovar 1A (n = 6).

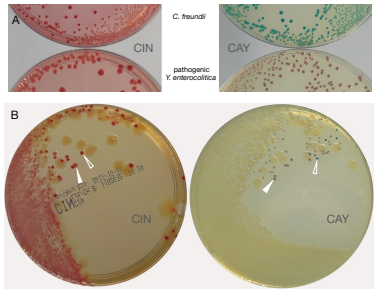

FIG. 4. Differentiation of pathogenic *Yersinia enterocolitica* from *Citrobacter freundii*, the most frequent false positive encountered on CIN in this study.
(A). Pure cultures on CHROMagar Yersinia (CAY, right) and CIN (left) after 48 h at 30°C
(B). Colonies plated out from a stool specimen containing *C. freundii*, *Morganella morganii* and pathogenic *Y. enterocolitica*. On CAY, mauve colonies of pathogenic *Y. enterocolitica* (plain arrowheads) can be readily differentiated from blue-green colonies of *C. freundii* (empty arrowheads). Incubation 48 h in air, 30°C.

- With almost 10-times less false positive results, the specificity of CAY on primary plating was therefore significantly higher than that of CIN ($P < 0.01$) (TABLE 3).

TABLE 3. Specificities of CIN and CAY media on primary plating of clinical stools specimens

Medium	No of true negative results	No of false positive results	Specificity (%)[a]
CIN	1,351	143[b]	90.42
CAY	1,479	15[c]	99.00

[a] (Number of true-negative results on the medium/number of negative samples) x 100.

[b] *Citrobacter freundii*, n = 88; *Providencia* spp., n = 35; *Aeromonas* spp., n = 12; *Yersinia enterocolitica* biovar 1A, n = 6; *Acinetobacter johnsonii*, n = 1; *Achromobacter xylosoxydans*, n = 1.

[c] *Stenotrophomonas maltophilia*, n = 9; *Brevundimonas terrae*, n = 3; *Shewanella putrefaciens*, n = 1; *Yersinia bercovieri*, n = 1.

Summary

CHROMagar Yersinia is a new chromogenic medium allowing specific recognition of **pathogenic** *Y. enterocolitica.*

Isolates of the pathogenic serotypes encountered in clinical practice all yielded typical mauve colonies when plated on CAY, in contrast to all avirulent isolates tested which grew as blue colonies.

The main false positives on CAY were *S. maltophilia* and *Y. bercovieri,* both infrequently encountered in clinical stool analysis. By contrast, false positives were almost 10 times more often present on CIN agar, the reference medium for *Y. enterocolitica* detection in stools.

Based on its significantly higher specificity (99,0% *versus* 90.4% for CIN, $P < 0.01$) and identical sensitivity, CAY can be recommended for routine detection of pathogenic *Y. enterocolitica* in human diarrheic stools.

References

Bottone, E. J., H. Bercovier, and H. H. Mollaret. 2004. *Yersinia. in* Bergey's Manual of Systematic Bacteriology. G. Garrity, Ed. Springer. **2B:**838-48.

Lantz, P. G., R. Knutsson, Y. Blixt, W. Abu Al-Soud, E. Borch, and P. Rådström. 1999. Detection of pathogenic *Yersinia enterocolitica* in enrichment media and pork by a multiplex-PCR: a study of sample preparation and PCR-inhibitory components. Int. J. Food Microbiol. 45:93-105.

Wannet, W. J. B., M. Reesink, H. A. Brunings, and H. M. E. Maas. 2001. Detection of pathogenic *Yersinia enterocolitica* by rapid and sensitive duplex assay. J. Clin. Microbiol. **39:**4483-86.