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Comparison of Four Chromogenic Media and Hektoen Agar for Detection and Presumptive Identification of *Salmonella* Strains in Human Stools

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Several chromogenic media have been developed to enhance the specificity of *Salmonella* detection. We compared the performance of four commercial chromogenic media—namely, ABC medium (Lab M. Ltd., Bury, United Kingdom), COMPASS Salmonella agar (Biokar Diagnostics, Beauvais, France), CHROMagar Salmonella agar (CHROMagar Company, Paris, France), and SM ID agar (bioMerieux, Marcy l'Etoile, France)—with conventional Hektoen medium. Nine hundred sixteen stool samples from inpatients at three hospitals were cultured, in parallel, on the five media, both by direct inoculation and after selective enrichment in selenite broth. Sixty-four *Salmonella* strains with 12 serotypes were isolated on at least one medium. After 48 h of incubation, sensitivity before and after enrichment was 62.5 and 89.1% with ABC medium, 77.1 and 93.8% with COMPASS agar, 66.7 and 89.1% with CHROMagar, 68.8 and 85.9% with SM ID agar, and 85.4 and 98.4% with Hektoen agar, respectively. Broth enrichment and prolonged incubation (48 versus 24 h) increased the sensitivity of all five media. Only one strain was not isolated on Hektoen agar. The number of false-positive isolates was higher with all five media after enrichment in selenite broth and after incubation for 48 h compared to 24 h. The specificity of the four chromogenic media was better than 91% after incubation for 24 h (77.7% with Hektoen agar) and better than 84% after incubation for 48 h (74.8% with Hektoen agar). This higher specificity reduces the need for confirmatory tests, thereby cutting technical time and reagent requirements. Both COMPASS agar and CHROMagar Salmonella, which after simple additional tests showed close efficiencies (96 and 97%, respectively), can be recommended as single-plate media of choice for the detection and presumptive identification of salmonellae in stools.

Conventional methods for *Salmonella* spp. detection in stools are based on nonselective and selective enrichment, followed by biochemical and serological identification. Conventional selective media for *Salmonella* isolation have very poor specificity, and the numerous false-positive results necessitate time-consuming complementary tests.

Two main approaches to *Salmonella* colony identification have been developed in the last decade: (i) screening tests applied to *Salmonella*-like colonies on common selective media, such as the C8 esterase test (2, 5, 9, 16, 18, 20, 27) and the OBIS Salmonella test (13; A. M. Freydiere, J. M. Perez, P. Cavalli, and V. Raverot, Abstr. 101st Gen. Meet. Am. Soc. Microbiol., abstr. C 173, p. 189, 2001), and (ii) chromogenic media.

Several agar media containing chromogenic substrates for *Salmonella*-specific enzymes have recently been developed. Rambach agar (9, 26) and *Salmonella* detection and identification medium (SM ID agar) (6, 17; M. C. Poupart, M. Mounier, F. Denis, J. Sirot, C. Couturier, and F. Villeval, Abstr. 5th Eur. Congress Clin. Microbiol. Infect. Dis., abstr. 1254, 1991) were the first media of this type. Rambach agar uses a chromogenic substrate for β-galactosidase (X-Gal), in conjunction with propylene glycol, which is fermented by *Salmonella* spp. to generate acid (26). SM ID agar is based on similar principles and incorporates X-Gal and glucuronic acid (Poupart et al., 5th ECCMID). SM ID agar is less specific than Rambach agar (6, 17), but Rambach agar fails to detect *Salmonella enterica* serovar Typhi and *S. enterica* serovar Paratyphi A (9, 26). ABC medium, a more recent product, uses a combination of two chromogenic substrates to detect salmonellae on the basis of their alpha-galactosidase production (23). Finally, several chromogenic media have been produced to detect *Salmonella* esterase activity (4, 10, 22; C. Roure, J. M. Perez, P. Cavalli, and A. M. Freydiere, Abstr. 101st Gen. Meet. Am. Soc. Microbiol., abstr. C 170, p. 188, 2001).

The purpose of this study was to assess the performance of four commercial chromogenic media—namely, ABC medium, COMPASS Salmonella agar, CHROMagar Salmonella agar, and SM ID agar—in comparison with conventional Hektoen agar for *Salmonella* detection in human stool specimens. We also examined the value of a preenrichment step and prolonged incubation.

(This work was presented in part [results for 539 stool samples] at the 101st General Meeting of the American Society for Microbiology.)

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MATERIALS AND METHODS

Stool specimens. From September 1999 to April 2002, 916 stool samples from inpatients at three hospitals (Hôpital de l'Antiquaille and Hôpital de La Croix-

TABLE 1. Principles of the chromogenic plates evaluated in this study for detection and presumptive identification of *Salmonella* species and colony colors of *Salmonella* and other *Enterobacteriaceae*

Medium (manufacturer)	Explanation of colony colors (enzyme detected [substrate used])	References
ABC (Lab M. Ltd.)	*Salmonella* yield blue colonies (α-galactosidase [5-bromo-4-chloro-3-indolyl-α-D-galactopyranoside]); other *Enterobacteriaceae* yield black colonies (β-D-galactosidase [3,4-cyclohexeno esculetin-β-D-galactoside]).	19, 23, 24
COMPASS Salmonella agar (Biokar Diagnostics)	*Salmonella* yield magenta colonies (esterase [5-bromo-6-chloro-3-indolyl-caprylate]); other *Enterobacteriaceae* yield blue colonies (β-glucosidase [5-bromo-4-chloro-3-indolyl-glucopyranoside]).	22, Roure et al.[a]
CHROMagar Salmonella agar (CHROMagar Microbiology)	*Salmonella* yield mauve colonies (esterase [patented substrate]); other *Enterobacteriaceae* yield blue or white colonies (β-D-galactosidase [patented substrate]).	6, 10, 15
SM ID agar (bioMerieux)	*Salmonella* yield pink colonies (D-glucuronic acid metabolization [neutral red acidification]); other *Enterobacteriaceae* yield blue or green colonies (β-D-galactosidase [5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside]).	5, 17, 28

[a] 101st Gen. Meet. Am. Soc. Microbiol.

Rousse, Lyon, France, and Hôpital de Pointe à Pitre, Pointe à Pitre, Guadeloupe, France) were prospectively tested. Patients comprised 66% infants and children (0 to 1 year, 25%; 1 to 15 years, 41%) and 34% adults, and all patients were admitted with a complaint of diarrhea or with unexplained fever.

A total of 543 specimens were studied at the Pointe à Pitre hospital, and 373 were studied at the Antiquaille hospital, using exactly the same protocol for inoculation, interpretation of cultures, and identification. (same conventional method, same biochemical test panels, same method of inoculation, and same reading procedure).

Media. Four commercial chromogenic media (Table 1)—namely, ABC medium (Lab M. Ltd., Bury, United Kingdom), COMPASS Salmonella agar (Biokar Diagnostics, Beauvais, France), CHROMagar Salmonella (CHROMagar Company, Paris, France), and SM ID agar (bioMerieux, Marcy l'Etoile, France)—were used as recommended by the manufacturers. These media are claimed to allow the detection and identification of salmonellae. Specific enzymes are detected by their effect on chromogenic substrates incorporated in the agar, and rapid tests (microscopic examination and oxidase activity) are applied directly to colonies to assist identification.

Hektoen enteric agar (bioMerieux) was the standard primary plating medium used for routine screening for *Salmonella* and *Shigella* spp. in the three participating laboratories.

Medium preparation. SM ID and Hektoen agar plates were supplied ready to use. The other three chromogenic media were supplied as powders in preweighed batches and were prepared according to the manufacturers' instructions in the different participating laboratories.

Each batch of medium was tested for sterility, culture response, and chromogenic reactions with the following reference strains: *Salmonella* serovar Enteritidis ATCC 13076, *Salmonella* serovar Typhimurium ATCC 14028, and *Enterobacter aerogenes* ATCC 13048. Plates were stored at 4°C in the dark for up to 4 weeks.

Selenite broth (bioMerieux) was used for sample enrichment.

Inoculation. Stool specimens were streaked in a single session onto the five media, both directly and after selective enrichment in selenite broth, as follows. Briefly, 1 g of solid stool (or 1 ml of liquid stool) was suspended in 10 ml of 0.85% saline, and the same amount of the same specimen was inoculated into selenite broth. Ten microliters of each suspension was streaked, with a calibrated loop, onto each medium. After incubation for 24 h at 36°C (enrichment procedure), 10 μl of selenite broth was streaked in the same way. All plates were incubated for 24 and 48 h at 36°C.

Readings. The Hektoen agar plates and the chromogenic plates were read by two independent technicians, after 24 then 48 h.

On Hektoen agar, blue-gray colonies with a black center (lactose negative, H_2S positive) and blue-green colonies (lactose negative) were identified biochemically as described below.

On the four chromogenic media, each colony corresponding to a *Salmonella* sp., according to the respective manufacturer's definitions (see Table 1), was subcultured as described below.

A false-positive colony was a colony that looked like *Salmonella* on the agar plate but that was not *Salmonella* upon further biochemical characterization.

Reference identification. Each suspected *Salmonella* strain isolated on the four different chromogenic agar plates was picked and subcultured in one of the four compartments of an SM ID agar plate. When the color and morphology of the colonies in the four compartments were similar, the colonies from a single chromogenic medium were fully identified by biochemical testing. When the colonies in the four compartments differed, each was subjected to biochemical identification.

Thus, for each suspected *Salmonella* strain isolated either on Hektoen agar or on the chromogenic media, a reference identification procedure was performed by standard biochemical methods (API 20E strip [bioMerieux] for *Enterobacteriaceae* and API 20NE strip [bioMerieux] for nonfermenters), and isolates confirmed as salmonellae were identified serologically by agglutination (Bio-Rad, Marnes la Coquette, France).

Statistical analysis. The sensitivity and specificity of the different media were analyzed with McNemar's test as recommended by Ilstrup (12).

RESULTS

***Salmonella* detection.** Sixty-four *Salmonella* strains were isolated on at least one medium and corresponded to 12 serotypes (Tables 2 and 3): serotype Areclavanetz ($n = 2$), serotype Enteritidis ($n = 27$), serotype Hadar ($n = 3$), serotype Houtenae ($n = 3$), serotype Infantis ($n = 2$), serotype Mississippi (n

TABLE 2. Comparison of media for detection of *Salmonella*, before enrichment and after 24 and 48 h of incubation, from 916 stool specimens from inpatients of three hospitals

Medium	No. of strains detected before enrichment									
	Salmonella		False positives		Sensitivity (%)		Specificity (%)		Efficiency (%)	
	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h	24 h	48 h
ABC	24	30	8	11	50.0	62.5	99.1	98.7	97	97
COMPASS agar	26	37	16	109	54.2	77.1	98.1	87.4	96	87
CHROMagar Salmonella agar	22	32	30	112	45.8	66.7	96.5	87.1	94	86
SM ID agar	25	33	54	80	52.1	68.8	93.6	90.8	92	90
Hektoen	30	41	127	159	62.5	85.4	85.1	81.7	84	82

A total of 48 isolates were recovered on at least one medium before enrichment (sensitivity, 100%)

TABLE 3. Comparison of five selective media for detection of *Salmonella*, after enrichment and 48 h of incubation, from 916 stool specimens from inpatients of three hospitals

Medium	No. of strains detected after enrichment and incubation for 48 h			Sensitivity[b] (%)	Specificity (%)	Specificity after CT (%)	Efficiency without CT (%)	Efficiency after CT (%)
	Positives	False positives	False positives after CT[a]					
ABC	57	17	10	89.1	98	98.8	97	98
COMPASS agar	60	108	33	93.8	87.3	96.1	88	96
CHROMagar Salmonella agar	57	130	22	89.1	84.7	97.4	85	97
SM ID agar	55	83	52	85.9	90.3	93.6	90	92
Hektoen	63	215	151	98.4	74.8	82.3	76	83

[a] CT = complementary tests. CT tests included microscopic examination to exclude yeasts and osidase testing to exclude pseudomonads.
[b] A total of 64 isolates were recovered on at least one medium after enrichment (sensitivity 100%)

= 1), serotype Mons (n = 2), serotype Panama (n = 16), serotype Paratyphi A (n = 1), serotype Rubislaw (n = 1), serotype Typhimurium (n = 5), and serotype Virchow (n = 1).

Salmonellae were detected in 2 of the 373 stools tested in Lyon (0.5%) and in 62 of the 543 stools tested in Guadeloupe (11.4%).

An enumeration of the *Salmonella* colonies performed on the different positive stools showed that 8% of the positive stools yielded $<10^4$ CFU/ml and might have been undetected by direct plating of 10 μl per plate after the initial 1:10 dilution of the fecal material.

Enrichment and prolonged incubation (48 h versus 24 h) increased the sensitivity of all the media tested (64 versus 48 isolates in total).

Salmonella isolates were subsequently streaked on the media on which they were not initially detected, and the expected colonial appearance was consistently obtained on these subcultures. The serovar did not appear to influence the sensitivity of any of the media tested. Interestingly, however, one serovar Enteritidis strain that was isolated before enrichment on four of the five media (not on ABC medium) was not isolated on any of the media after enrichment.

Without enrichment, after 24 h of incubation, between 45.8 and 62.5% of all the *Salmonella* isolates were detected, according to the medium. Hektoen agar was significantly more sensitive than the other four media (62.5 and 85.4% after incubation for 24 and 48 h, respectively), while the sensitivities of the four chromogenic media were not significantly different from each other ($P > 0.05$).

With enrichment and a 48-h incubation period, Hektoen agar again had the highest sensitivity (98.4%), although the sensitivity of COMPASS agar (93.8%) was not significantly different ($P = 0.361$). SM ID agar had the lowest sensitivity (85.9%).

Specificity. The specificity of the four chromogenic media after incubation for 24 h was 91%, compared to 77.7% with Hektoen agar (Table 2). With enrichment and a 48-h incubation period (Table 3), ABC medium had the highest specificity (98%; $P > 0.0001$) and Hektoen agar had the lowest specificity (74.8%). The difference in specificity between COMPASS agar and CHROMagar Salmonella was not significantly different ($P = 0.124$). Except for ABC medium, prolongation of the incubation period from 24 to 48 h considerably increased the number of false-positive results, especially with COMPASS agar (from 16 to 109) and CHROMagar Salmonella agar (from 30 to 112).

The enrichment procedure increased the number of false-positive isolates (other than coliforms) on Hektoen medium and partially inhibited yeasts on COMPASS agar, CHROMagar Salmonella agar, and SM ID agar. The false-positive rates obtained with the different media after incubation for 48 h are reported in Table 4. With COMPASS agar and CHROMagar Salmonella agar, the two main genera yielding false-positive results were *Candida* and *Pseudomonas.* Both were easily ruled out by complementary tests (direct examination for *Candida* and instantaneous oxidase test for pseudomonads) (Table 3).

The best overall performance among the five media according to a measure of efficiency (Tables 2 and 3) was shown by ABC medium, which was closely followed by COMPASS agar and CHROMagar Salmonella agar.

DISCUSSION

Some new chromogenic media for the detection and presumptive identification of salmonellae have been compared individually with conventional media (7, 10, 15, 23). The results of these studies are difficult to compare with each other, owing to the use of various reference media and different strain and sample panels. We therefore compared the performance of four chromogenic media and conventional Hektoen medium in the same conditions and with the same clinical stool specimens, with and without selective enrichment in selenite broth.

The final *Salmonella* isolation rates in Lyon (0.5%) and Guadeloupe (11.4%) were similar to those obtained in previous studies in the same areas with undiluted stools (1, 17), showing that the dilution of the stools did not influence the overall performance of the media.

Although 8% of the *Salmonella* spp. strains might have not been detected by direct plating due to our methodology, the review of two recent studies comparing CHROMagar Salmonella agar and Hektoen agar demonstrates that the inoculum size cannot explain solely the variability of the direct plating sensitivities from one study to another. As a matter of fact, with 27 *Salmonella* strains isolated, Eigner et al. (7), who used an undefined inoculum, reported direct plating sensitivities of 85 versus 59%, respectively. With 20 *Salmonella* isolates, Gaillot et al. (10), using an inoculum of 50 μl of liquid stool or of stool liquefied in saline solution, reported direct plating sensi-

TABLE 4. Distribution of non-*Salmonella* genera yielding *Salmonella*-like colonies on different media before and after enrichment and after 48 h incubation[a]

Species	No. of strains detected before and after enrichment and incubation for 48 h									
	ABC medium		COMPASS agar		CHROMagar-Salmonella agar		SM ID agar		Hektoen agar	
	BE	AE	BE	AE	BE	AE	BE	AE	BE	AE
Pseudomonas spp.	7	7	15	37	30	57	16	26	45	63
Yeasts	0	0	60	38	67	51	13	5	2	1
Nonfermenting bacilli	0	0	0	0	0	0	0	0	2	0
Citrobacter spp.	0	1	0	0	0	1	6	12	29	45
Klebsiella, *Enterobacter*, and *Serratia*	3	2	0	3	0	3	8	3	12	17
E. coli	1	0	13	8	4	2	13	6	19	8
Proteae	0	3	11	11	10	13	19	19	46	77
Unidentified bacilli	0	4	9	11	1	3	5	12	4	4
Shigella spp.	0	0	1	0	0	0	0	0	0	0
Total no. of false-positive isolates after 48 h of incubation	11	17	109	108	112	130	80	83	159	215

[a] Abbreviations: BE, before enrichment; AE, after enrichment in selenite broth.

tivities of 95 versus 80%, respectively. With 69 *Salmonella* strains isolated, this present study using an inoculum of 10 μl of an initial 1:10 dilution of the fecal material reports direct plating sensitivities of 66.7 versus 85.4% for CHROMagar Salmonella and Hektoen agars, respectively. Moreover, this variability of the results from one study to another demonstrates the necessity to standardize the different methodologies in the future.

In contrast to other authors (7, 10, 15, 17), we found that the chromogenic media tested here (except for COMPASS agar) were less sensitive than Hektoen agar. Some of the *Salmonella* isolates in our study may not have expressed the specific enzyme activities detected by the chromogenic media. This was the case in a recent study performed in Burkina Faso (24), in which nine strains (including strains belonging to the two most commonly isolated serovars, namely, Enteritidis and Typhimurium) out of 20 *Salmonella* strains isolated on ABC medium did not express alpha-galactosidase activity. These results also suggest that the enzyme expression by salmonellae may vary according to the geographical origin of the strain. However, in our study, all the isolates generated the specific colony color after subculture on the different media. Competing flora may also affect the sensitivity of chromogenic media. Recently, Nye et al. (19) showed that the sensitivity of ABC medium was adversely affected by even small amounts of competing flora. In contrast, species discrimination by the chromogenic media tested here was considerably better than with Hektoen agar.

Our results underline the importance of sample enrichment prior to plating and of prolonged incubation (48 versus 24 h) for recovery of salmonellae on both conventional and chromogenic media (3; Roure et al., 101st Gen. Meet. Am. Soc. Microbiol.). Forward and Rainnie (8) and Kelly et al. (14) recently reported similar results and recommended that selenite enrichment broth be used systematically to maximize sensitivity for serovar Enterica.

Forward and Rainnie advised against direct plating and recommended inoculating only selenite enrichment broth, on day 1 (8). However, enrichment broth may be toxic for some *Salmonella* strains (21), as was the case for one of the isolates found in this study. Moreover, direct plating identified serovar Enterica in about 50% of cases, 1 day earlier than would have been possible if only selenite enrichment broth had been inoculated on day 1.

The higher specificity of chromogenic media compared to Hektoen agar confirms previous findings (7, 10, 23, 28). This reduces the need for biochemical identification and thereby saves technical time and reagent requirements.

As in previous studies (7, 10, 15; Roure et al., 101st Gen. Meet. Am. Soc. Microbiol.), the two main microorganisms yielding false-positive results on CHROMagar Salmonella and COMPASS agars were *Candida* spp. and pseudomonads. Although these genera can readily be distinguished from salmonellae by microscopic examination and rapid oxidase testing, respectively, the addition of antifungal and antipseudomonal agents to these media might increase their specificity. Recently, Eigner et al. (7) tested a modified CHROMagar Salmonella formula containing cefsulodin and amphotericin B and found that pseudomonads and yeasts were effectively inhibited. Similar results have also been obtained in our laboratory with a new formula of COMPASS agar containing antifungal and antipseudomonal agents (data not shown).

Except for ABC medium, which detects alpha-galactosidase activity, all the chromogenic media recently developed for presumptive *Salmonella* identification detect esterase activity (4, 10, 22; Roure et al., 101st Gen. Meet. Am. Soc. Microbiol.). Detection of esterase activity has been widely used in spot tests applied directly to colonies (e.g., the MUCAP test from Biolife and a home-made C8 esterase test) (2, 5, 9, 16, 18, 20, 27) and in panels such as API Z and Rapidec Z (bioMerieux) (11, 25, 29; D. Monget, 8 May 1980, United Kingdom patent application 2,050,418). However, detection of this activity on agar medium during bacterial growth was hindered by technical difficulties (22). The sensitivities of CHROMagar Salmonella agar (7, 10) and COMPASS agar (Roure et al., 101st Gen. Meet. Am. Soc. Microbiol.) are better than those of other chromogenic media, such as Rambach agar, which does not detect serovars Typhi or Paratyphi A.

Most chromogenic media have been designed to detect fre-

quently isolated microorganisms such as *E. coli* (50 to 80% of urinary tract pathogens) and *Candida albicans* (60% of human pathogenic fungi). In contrast, chromogenic media for presumptive identification of salmonellae are mainly designed to exclude the numerous microorganisms with similar colony characteristics. Thus, such media must above all be selective and specific. In our hands, ABC medium had the best diagnostic performance of the four chromogenic media tested. However, the use of this medium is restricted by the recent detection of *Salmonella* strains which do not produce alpha-galactosidase activity (24) and the adverse effect of the competing flora on sensitivity (19).

Although we did not conduct a comparative cost analysis, the chromogenic media tested here, before enrichment and with a 24-h incubation period, identified 50% of all the *Salmonella* isolates, with a specificity exceeding 93%, thereby reducing the need for confirmatory tests and providing rapid and economic screening. Moreover, although there was a noteworthy decrease in the specificity of some chromogenic media after enrichment and prolonged incubation (48 h), all the chromogenic media still had higher specificities than Hektoen agar after 48 h and therefore remained more economical than the latter medium.

Although ABC medium showed the best diagnostic performance of the four chromogenic media tested in this study (efficiency of 98%), the high prevalence of strains which did not produce alpha-galactosidase in some geographical locations (24) placed limitations on the use of this medium. Thus, both COMPASS agar and CHROMagar Salmonella agar, which after simple additional tests showed close efficiencies (96 and 97%, respectively), can be recommended as single-plate media of choice for *Salmonella* detection and presumptive identification.

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