Comparison of *Salmonella* Chromogenic Medium with DCLS Agar for Isolation of *Salmonella* Species from Stool Specimens

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Salmonella chromogenic medium (SCM; Oxoid, Basingstoke, United Kingdom), a new selective chromogenic medium, was compared to DCLS agar (Oxoid) for the detection and presumptive identification of *Salmonella* species from stool samples. This medium contains two chromogenic substrates, Magenta-cap (5-bromo-6-chloro-3-indolylcaprylate), which is hydrolyzed by *Salmonella* species to give magenta colonies, and X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside), which is incorporated to visualize β-D-galactosidase-producing organisms as blue colonies. Thus, non-*Salmonella* organisms appear blue or are not stained by any of the chromogens of the medium. A total of 500 stool samples were investigated by plating them directly and after selenite enrichment on DCLS agar and SCM. A total of 44 *Salmonella*-positive stool samples were detected. The sensitivities for direct plating and after enrichment were 22.7 and 81.8%, respectively, for DCLS agar, and for SCM these values were 34.1 and 100%, respectively. The specificities for direct plating and after enrichment were 82.5 and 72.8%, respectively, for DCLS agar and 98.5 and 95.8%, respectively, for SCM. According to these results, the sensitivities of SCM and DCLS agar were comparable on primary plating. However, the sensitivity of SCM was significantly higher after enrichment. In addition, the specificity of SCM was also significantly higher than that of DCLS agar both before and after enrichment. On the basis of these results, SCM can be recommended for the isolation of *Salmonella* species from stool samples in preference to DCLS agar.

Salmonellosis continues to be a major health problem, and its diagnosis most often involves direct detection of bacteria in stools by culture or, more recently, by PCR after enrichment (11, 19, 21). Isolation of *Salmonella enterica* on selective culture media is different from PCR in that it allows further identification of the bacteria and antibiotic susceptibility testing, which are important for disease control (8). Thus, a wide variety of selective differential agars have been developed for this purpose. The selective agents used in these agars include dyes, antibiotics, and bile salts (7). Differentiation of most salmonellae from other organisms usually relies on the visualization of simple biochemical features such as the production of hydrogen sulfide or the nonfermentation of lactose (2, 3, 6, 16; D. E. Post, Folio LT0435A [1997], Oxoid, Ltd., Basingstoke, United Kingdom). These media are useful for the confirmation of suspect colonies but most are highly nonspecific, mainly due to *Proteus* and *Citrobacter* strains from the normal flora, which closely resemble *Salmonella* strains. Such suspect colonies must be differentiated further by biochemical or serological tests before a final result is obtained (5, 6, 10, 20). Due to high rates of false-positive results, screening of stool samples for *Salmonella* becomes labor-intensive, with additional costs for subsequent identification (5). Such a medium is DCLS agar, which is used for the detection of *Salmonella* species in our microbiology laboratory. This medium is a modification of a formula described by Leifson (12) in 1935 for the isolation of enteric pathogens. DCLS agar is a derivative of desoxycholate citrate agar and contains sucrose, as well as lactose, to improve the differentiation of salmonellae from

Proteus spp. and other lactose-negative, sucrose-positive bacteria (D. E. Post, Oxoid, Ltd.).

In the last decade chromogenic media have been developed for the detection of *Salmonella* species (2, 4, 6, 18). These media use a combination of chromogenic substrates and conventional biochemical tests and are, therefore, highly specific. This reduces the workload with regard to unnecessary examination of suspect colonies, saving time, supplies, and money (13, 14, 16). Chromogenic enzyme substrates are compounds which act as the substrate for specific enzymes and change color due to the action of the enzyme (14). The first medium of this type was Rambach agar. *S. enterica* produces acid from propylene glycol present in the medium to give a red color due to the neutral red indicator. In addition, it incorporates the chromogenic substrate X-Gal (5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside) that is hydrolyzed by many non-*Salmonella* species to yield blue colonies (18). Rambach agar is highly specific; however, it has a disadvantage in that it does not detect *S. enterica* serovars Typhi and Paratyphi A (1, 5, 9). Another chromogenic medium similar to Rambach agar is SM-ID agar, which uses two chromogenic substrates for β-galactosidase (i.e., X-Gal) and β-glucosidase (i.e., X-Glu). These substrates allow differentiation of *Salmonella* (negative) from other members of the family *Enterobacteriaceae* (positive), which yield a blue to purple color. *Salmonella* strains produce acid from glucuronate present in the medium to yield a red color due to the neutral red indicator (13, 14). This medium also detects *S. enterica* serovars Typhi and Paratyphi (5, 15).

More recently, a new chromogenic medium for the detection of *Salmonella* species, *Salmonella* chromogenic medium (SCM), has been developed. This medium utilizes an optimized nutritious base medium, which is a DCLS-type medium. It contains two chromogenic substrates, which were chosen to ensure high

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TABLE 1. Serovars isolated from the stool samples examined
by using the two selective media

Salmonella serovar	No. of isolates detected with:		
	Any medium	DCLS agar	SCM
Kentucky	2	1	2
Enteritidis	26	21	26
Infantis	4	2	4
Virchow	2	2	2
Typhimurium	3	3	3
Hato	2	2	2
Nima	2	2	2
Agona	2	2	2
Give	1	1	1
Total	44	36	44

specificity of the medium (Oxoid, Ltd. [press release]). The first substrate, Magenta-cap (5-bromo-6-chloro-3-indolylcaprylate) is hydrolyzed by *Salmonella* species to give magenta colonies. The second substrate, X-Gal, is incorporated to visualize β-D-galactosidase-producing organisms as blue colonies (N. L. Lang, A. Mari, and J. A. M. Lees, Oxoid, Ltd. [unpublished data]; T. Sadler, J. Raggett, and D. Coleman, Oxoid, Ltd., poster LT0759A, 2001). Thus, most competitive colony growth is blue on this medium. Other colonies, which do not utilize the chromogens, grow as colorless colonies (Sadler et al., poster). The colors produced are strong, easily distinguished, and unlike some chromogenic media, do not fade (Sadler et al., poster; Oxoid, Ltd. [press release]; Lang et al., unpublished data). Compared to many of the traditional media, which rely on H_2S production, and/or the nonfermentation of lactose, SCM isolates the H_2S-negative and/or lactose-fermenting strains. Thus, *Salmonella* species detected with this medium include biochemically atypical strains such as the *S. enterica* serovars Typhi, Paratyphi, and Choleraesuis and the nonmotile *Salmonella* serovar Gallinarum (Oxoid, Ltd. [press release]).

The selective agents present in SCM include bile salts, which inhibit the growth of gram-positive bacteria, and the use of two antibiotics: novobiocin, which inhibits the growth of *Proteus* spp., and cefsulodin, which inhibits the growth of *Pseudomonas* spp. (both from Oxoid, Ltd.).

The purpose of the present study was to compare the sensitivity, specificity, and cost-effectiveness of SCM to DCLS agar for the isolation of *Salmonella* species from stool specimens.

MATERIALS AND METHODS

Culture media. SCM and DCLS agar were obtained from Oxoid, Ltd., Basingstoke, United Kingdom. They were supplied in powder form and prepared according to the manufacturer's instructions. All media were subjected to full quality control procedures, and performance standards were verified by using American Type Culture Collection strains obtained from Oxoid. Prepared media were stored in a refrigerator and used within 14 days of preparation.

Clinical samples. The study was carried out between June and September 2002 at the Microbiology Laboratory in St. Luke's Hospital, G'Mangia, Malta, with 500 routine fecal samples received from both hospitalized and nonhospitalized patients. Approximately 1 g of formed fecal material was emulsified in 3 ml of tryptone water (Oxoid). Liquid stool samples were diluted 1 in 4 (vol/vol) in tryptone water. Then, 50 μl of fecal suspension was inoculated onto SCM and DCLS agar plates and spread with sterile loops for single colonies. Concurrently, 1 ml of fecal suspension was inoculated into selenite broth to be incubated overnight at 37°C and then subcultured onto SCM and DCLS agar plates (50 μl per plate). All plates were incubated for 18 to 24 h at 37°C aerobically.

Presumptive identification. All plates were read by the same technologist. Suspicious isolates were defined as follows: magenta colonies on SCM and pale, translucent, or colorless colonies on DCLS agar.

SCM protocol. Magenta colonies on SCM were subcultured onto a nutrient agar plate and incubated for 18 to 24 h at 37°C aerobically. Colonies were first tested for their oxidase activities on pieces of filter paper impregnated with *N*,*N*,*N*,*N*-tetramethyl-1,4-phenylenediamine-dihydrochloride (Sigma, St. Louis, Mo.). Only oxidase-negative colonies were processed further. These were identified by using ATB ID 32E strips (bioMerieux, Marcy l'Étoile, France). *Salmonella* isolates were serotyped after overnight culture on nutrient agar slopes (Oxoid) by agglutination with anti-O and anti-H antisera (SIFIN, Berlin, Germany) by using the Kauffmann-White scheme (17). Spontaneous agglutination was ruled out by testing the isolates with saline without the antisera. MacConkey agar (Oxoid CM507) was used as a purity plate.

DCLS protocol. Pale, translucent, or colorless colonies on DCLS medium were subcultured onto both a urea agar slope and a MacConkey purity plate and incubated for 18 to 24 h at 37°C in air. Urease-negative colonies were then identified further by using ATB ID 32E strips and serology (as in the SCM protocol).

Statistical analysis. Sample size was determined by using comparison of proportions. The differences in the sensitivities and specificities of the two media were analyzed by using the McNemar test.

RESULTS

From 500 stool specimens examined, 44 strains of *Salmonella* were isolated on at least one medium, yielding a positivity rate of 8.8%. The distribution of serovars is shown in Table 1. Upon primary plating, 15 *Salmonella* strains were detected on SCM and 10 *Salmonella* strains were detected on DCLS agar, yielding sensitivities of 34.1 and 22.7% for SCM and DCLS, respectively (Table 2). This difference is not statistically significant ($P = 0.181$). In this direct culture, seven *Salmonella* strains were detected by SCM only, and two strains were detected by DCLS agar only. After enrichment, all *Salmonella* isolates grew on SCM and 36 isolates grew on DCLS agar; thus, the sensitivities were increased to 100 and 81.8% for SCM and DCLS, respectively (Table 2). This difference is statistically significant ($P = 0.013$). Hence, after enrichment, eight *Salmonella* strains were detected with SCM only, and none were detected with DCLS only. All of the *Salmonella* strains detected on primary plating by any medium were also detected after enrichment. *Salmonella* strains which were not isolated in a particular medium grew with their characteristic colony color after inoculation of the pure strain into the medium in question. There was no apparent reduction in the number of colonies recovered when nutrient agar was used as a control. This confirmed that the failure in recovery was not due to the inhibitory effect of the medium or to growth of atypical colonies.

As shown in Table 3 the differences in the specificities of

TABLE 2. Recovery of *Salmonella* serovars by using the two
selective media before and after enrichment in selenite broth[a]

Medium	No. of true-positive isolates	Sensitivity (%)[b]
DCLS (primary plating)	10	22.7
SCM (primary plating)	15	34.1
DCLS (after enrichment)	36	81.8
SCM (after enrichment)	44	100

[a] A total of 44 isolates were recovered.
[b] The percent sensitivity was calculated as follows: (the number of true-positive results on medium/the number of positive samples) × 100.

TABLE 3. Specificities of the two selective media
before and after enrichment

Medium	No. of true-negative results	No. of false-positive results	Specificity (%)[a]
DCLS (primary plating)	376	80	82.5
SCM (primary plating)	449	7	98.5
DCLS (after enrichment)	332	124	72.8
SCM (after enrichment)	437	19	95.8

[a] The percent specificity was calculated as follows: (the number of true-negative results on medium/the number of negative samples) × 100.

both media, before and after enrichment, were statistically significant. The specificities on primary plating were 98.5 and 82.5% for SCM and DCLS, respectively ($P < 0.0001$). After enrichment, the specificities of both media decreased to 95.8 and 72.8% for SCM and DCLS agar, respectively ($P < 0.0001$). The number of false-positive isolates was lower on SCM than on DCLS agar. A total of 26 false-positive isolates were detected on SCM (7 from primary plating and 19 following enrichment), whereas 204 false-positive isolates were detected on DCLS agar (80 from primary plating and 124 after enrichment). The false-positive strains obtained from SCM consisted of *Enterobacter intermedius* (1 isolate), *Enterobacter amnigenus* (3 isolates), *Pseudomonas aeruginosa* (16 isolates), *Enterobacter cloacae* (1 isolate), *Pseudomonas putida* (1 isolate), *Acinetobacter baumannii* (1 isolate), *Aeromonas sobria* (1 isolate), *Escherichia coli* (1 isolate), and *Pantoea* species (1 isolate).

The use of screening strategies reduced the amount of organisms requiring further confirmatory testing, as shown in Table 4. Use of urea in the DCLS protocol eliminated 136 of 204 false-positive colonies detected on this medium, both before and after enrichment. A total of 114 ATB ID 32E strips were required to further identify urease-negative colonies. Of these 114 ATB ID 32E strips, 68 identified false-positive isolates. The oxidase test in the SCM protocol eliminated 18 of 26 false-positive colonies, and 67 ATB ID 32E strips were required to further identify presumptive *Salmonella* colonies detected, both before and after enrichment. Of these 67 ATB ID 32E strips, 8 identified false-positive isolates. The results showed that 69.4% of the presumptive *Salmonella* colonies on SCM were found to be positive compared to only 18.4% found to be positive on DCLS agar.

DISCUSSION

There are several selective plating media for the isolation of salmonellae from human feces and other specimens. Their specificity and sensitivity vary considerably. Several studies show that newly chromogenic media which have been developed for the detection of *Salmonella* species have a higher specificity than conventional media (4, 8, 15; Sadler et al., poster). Some are also reported to have a higher sensitivity (2, 6, 16).

In the present study, the sensitivities of SCM and DCLS agar were comparable on primary plating; however, the sensitivity of SCM was significantly higher than that of DCLS agar after enrichment. This may have resulted from a better separation of colonies on SCM, where there was an easy distinction of magenta from blue or colorless colonies. Eight *Salmonella*-posi-

tive stool samples were detected by SCM only; however, all of the relevant cultures on DCLS agar of these positive samples revealed presumptive *Salmonella* colonies. Therefore, *Salmonella* colonies may have been present but were mixed with a predominant growth of commensal organisms, which were indistinguishable from *Salmonella* species in their colonial appearance.

The specificity of SCM was superior to that of DCLS agar, both before and after enrichment, with significant statistical differences. Most false-positive cultures on SCM were due to *Pseudomonas* species, which accounted for 65.4% (17 isolates) of the total false positives, despite the presence of cefsulodin in the medium. A possible explanation for this could be a decrease in the activity of cefsulodin during the preparation of the medium, which may be due to the addition of the antibiotic before boiling. These colonies of *Pseudomonas* species were easily differentiated from *Salmonella* by the oxidase test.

The average current price of SCM plate is ca. 0.73 Euro; the DCLS agar plate is 0.12 Euro, Selenite broth bottle is 0.05 Euro, and tryptone water is 0.0013 Euro. For suspicious isolates on DCLS agar, a urea agar slope (average price, 0.04 Euro) and a MacConkey purity plate (average price, 0.06 Euro) were used. Urease-negative isolates were then identified biochemically using ATB ID 32E strips (average price, 2.38 Euro/strip). Thus, the total cost for analysis of the 500 stool specimens with the DCLS agar was 448.8 Euro. For suspicious colonies on SCM, a nutrient agar plate (average price, 0.03 Euro) was used. The oxidase-negative colonies isolated were further identified biochemically using ATB ID 32E strips (average price, 2.38 Euro/strip). Thus, the total cost for analysis of the 500 stool specimens with SCM was 921.7 Euro. These calculations do not take into account the labor time and the serological tests used to identify the *Salmonella* species. This result shows that the use of a chromogenic medium for the isolation of *Salmonella* from stool specimens has a significant impact on cost. However, this is offset by the substantially reduced labor time due to higher specificity than with conventional media, since staff costs contribute significantly to the total cost of running a diagnostic laboratory.

In conclusion, SCM has been shown to have good sensitivity and specificity. Interpretation of the colors is easy. Thus, there is a clear-cut differentiation of *Salmonella* strains from accompanying organisms, even when there is a low number of *Salmonella* colonies in mixed cultures. SCM is more specific than DCLS agar and, therefore, reduces considerably the workload in a diagnostic laboratory. The few false-positive cultures ob-

TABLE 4. Comparison of the number of ATB ID 32E strips used
on isolates from DCLS agar and SCM before and after enrichment

Group	No. of strips on:	
	DCLS agar	SCM
Total false positives	204	26
Urea-positive isolates (not tested)	136	0
Oxidase-positive isolates (not tested)	0	18
False positives tested	68	8
Salmonella-positive isolates	46	59
Total ATB ID 32E strips	114	67

tained on SCM were usually due to *Pseudomonas* species, which could be easily distinguished from *Salmonella* species by the oxidase test. Moreover, the good sensitivity of SCM makes the medium excellent for the detection and presumptive identification of salmonellae in the routine analysis of stool specimens. Further studies with SCM prepared with the antibiotics added after the medium is cooled to 50°C and then compared against the most commonly used media are required. Such an approach may result in improved selectivity and specificity of the medium.

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