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Efficiency of Plating Media and Enrichment Broths for Isolating *Salmonella* Species from Human Stool Samples: A Comparison Study

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Abstract

A comparative study was performed to evaluate best practice culture media and enrichment broths for recovering *Salmonella* species from human stool samples. A total of 1297 human stools were collected and processed in this study. Evaluation of agar media was carried out by direct plating (DP), 1096 stool samples were inoculated on Modified Semisolid Rappaport-Vassiliadis (MSRV), Xylose-Lysine-Deoxycolate (XLD), MacConkey (MAC), and Hektoen Enteric (HE) agars. Evaluation of enrichment broths were carried out by enrichment all 1297 stool samples in Selenite broth (SB), Rappaport-Vassiliadis (RV) broth, and Buffered Peptone Water (BPW), followed by plating on MSRV, MAC, and HE agars. A total of 102 *Salmonella*-positive stools by DP, 85.3% (87/102) were recovered utilizing MSRV while recovery from XLD, MAC, and HE agars were 34.3% (35/102), 34.3% (35/102), and 29.4% (30/102) respectively. A total 299/1297 stools samples were *Salmonella*-positive on at least one plating medium after enrichment procedure were 77.3% (177/299) for SB, 86.0% (197/299) and 78.6% (180/299) for RV and BPW respectively. All *Salmonella* isolated in this study was nontyphi *Salmonella*. Presently, the data suggest that the use of MSRV over MAC, HE, and XLD agars for isolation nontyphi *Salmonella* species from human stools is more efficacious. Additionally, use of MSRV in combination with MAC and HE agars following enrichment in RV broth enhances recovery of nontyphi *Salmonella* species. However, RV broth is inhibitory to typhi *Salmonella*, thus use of MSRV medium in combination with MAC, HE or XLD agars in direct plating following enrichment in non-selective BPW is an alternate method for recovery of both typhi and nontyphi *Salmonella* species contaminated in human stool samples.

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Keywords

Human Stool Specimens, Culture Media, *Salmonella*, Isolation, Enrichment

1. Introduction

Food-borne salmonellosis continues to be an important public health problem both in developed and developing countries [1]-[3]. Gastroenteritis is the most frequent illness derived from this infection and the use of stool or rectal swab samples are the main specimens used in diagnostic clinical laboratories. However, the presence of saprophytic flora, especially other coliforms in stool samples, has traditionally made isolation of *Salmonella* spp. difficult. Consequently, there is no ideal method for diagnosis of *Salmonella* infection and the development of highly selective media continues to be critical importance for diagnostic and treatment purposes. Conventional media base identification relies on the production of H_2S and pH indicator to detect lactose fermentation while bile salts and dyes inhibit saprophytic flora. However, these media types are inadequate to differentiate *Salmonella* spp. from other members of the Enterobacteriaceae, such as *Proteus* spp. and *Citrobacter* spp. [4]. In decreasing order of selectivity, MacConkey agar (MAC), Hektoen Enteric (HE) agar, *Salmonella*-Shigella (SS) agar, and Xylose-Lysine-Desoxycolate (XLD) agar are some of the more commonly used plating media in clinical laboratories.

Recently, several agar media containing chromogenic substrates for *Salmonella*-specific enzymes have been developed, *i.e.* Rambach agar [5], *Salmonella* Detection and Identification Medium (SM-ID; bioM'erieux, France), and ABC medium (Lab M. Ltd., Bury, United Kingdom). However, chromogenic agar media are less sensitive, but have greater specificity when compared to conventional media [6] [7]. Analternative method implementing enriched semisolid motility medium for isolating *Salmonella* from stool samples has been previously investigated. Jones & Handley used a semisolid medium containing cacotheline as a selective medium after initial culture of suspected material in a broth containing hydroquinone [8]. Ino & Graber used passage through semisolid medium in a "U" tube to recover *Salmonella* from cultures contaminated with *Pseudomonas aeruginosa* [9]. Stuart and Pivnick used selective motility (SM) medium modified with selenite F broth to make a semisolid medium contained in motility tubes to isolate *Salmonella* spp. from stool specimens. Data showed that the SM technique was equal or superior to those standard routine procedures employed in clinical laboratories and primary isolation of *Salmonella* from SM enrichment were relatively free from competing floras. Disadvantages to using the SM medium were the media was not shelf stable, could not be autoclaved and the migration time of *Salmonella* through the medium were lengthy [10].

Harper and Shortidge modified *Shigella-Salmonella* medium to a semisolid agar contained in Craigie tube to isolate *Salmonella* from stool specimens and found that 90% of specimens from which *Salmonella* were isolated were positive. Over 40% of these specimens would have been reported negative if the method had not been in use [11]. Modified Semisolid Rappaport-Vassiliadis (MSRV) medium is a modification of Rappaport-Vassiliadis enrichment broth for detecting motile *Salmonella* spp. in food products and feces [12] [13]. The original study on MSRV medium revealed a semisolid could be used as a rapid and sensitive method for isolation of *Salmonella* spp. from food products following pre-enrichment or selective enrichment [14] [15]. The efficacy of MSRV medium is based on the ability of *Salmonella* spp. to migrate through the selective medium ahead of other competing motile bacteria, thus producing opaque halos of growth surrounding the inoculating area. MSRV can be used as a plating medium for isolating *Salmonella* spp. (other than *S.* Typhi and *S.* Paratyphi A) from stool specimens with high sensitivity and specificity relative to chromogenic and conventional agar media [16] [17].

In order to evaluate the performance of MSRV, XLD agar media, and enrichment broths (e.g., BPW and RV) with our standard method for *Salmonella* spp. detection (MAC and HE agars and SB enrichment broth), a comparative study was conducted by direct plating for the isolation of *Salmonella* spp. Additionally, samples were enriched in SB broth (routine standard medium), BPW and RV broths, and prior to plating.

2. Materials and Methods

2.1. Specimens

The study was conducted from June 2003 to December 2004. Stool samples were collected from several diarr-

hea surveillance studies conducted by the Department of Enteric Diseases, Armed Forces Research Institute of Medical Sciences and processed for isolation of enteric bacteria pathogens.

2.2. Transport and Culture Media

Modified Cary-Blair transport medium (Approximate formula per liter, Sodium Thyoglycollate 1.5 g, Disodium Phosphate 1.1 g, Sodium Chloride 5.0 g, Agar 1.6 g). MAC agar (Difco, MD,USA), HE agar (Difco, MD,USA), MSRV (E. Merck, Darmstadt, Germany), Xylose-Lysine-Desoxycolate (XLD) agar, Selenite broth (SB) (BD Difco, USA), BPW (E. Merck, Darmstadt, Germany), and RV (R10 Broth, BD Difco, USA) were prepared according to the manufacturer's instructions. Prepared media for isolation and identification was tested with ATCC strains as quality control to assure quality of the prepared media.

2.3. Method

A total of 1297 stool samples were collected from both adults and children and were inoculated in SB, RV, and BPW enrichment broths prior to plating on MAC, HE, and MSRV. 1096 of the 1297 stools samples were used to compare the number of *Salmonella*-positive stool samples from MSRV, XLD, MAC, and HE agars by direct plating only with no enrichment step prior to plating.

2.4. Direct Plating Method

Stool suspensions (approximately 1:10 dilution) were prepared from fecal and rectal swabs by agitation of swabs in 0.85% sodium chloride solution and a drop of each stool suspension was used to inoculate MAC, HE, and XLD media and then streaked for growth of discrete colonies with a sterile loop. Inoculate 3 drops of stool suspension in separate spots on the surface of MSRV medium. MAC, HE, and XLD plates were incubated aerobically at 37˚C, for 18 - 24 hours, while MSRV media was incubated aerobically in an upright position at 42˚C, for 20 - 24 hours.

2.5. Enrichment

Approximately 0.5 mL of each stool suspension was inoculated into SB and BPW enrichment broths and incubated aerobically at 37˚C for 18 - 24 hours while inoculated RV broth was incubated at 42˚C for 18 - 24 hours, followed by subculture onto MAC, HE, and MSRV media and incubated as described above. Following the incubation period, MAC, HE, and MSRV media were examined for growth.

2.6. Identification

Up to three colonies suspected to be *Salmonella* spp. from each MAC, HE, and XLD agar media were inoculated onto KliglerIron agar (KIA), Lysine decarboxylase, Mannitol and Tryptone broth. Halos produced by swarming growth on MSRV medium were subcultured onto HE agar and incubated as described above. Suspected *Salmonella* isolates were confirmed by slide agglutination tests with O antisera for *Salmonella* according to the manufacturer's instructions (Polyvalent O antisera and individual group A—group 67, Serotest, S & A REAGENT LAB, Bangkok, Thailand).

2.7. Statistical Analysis

The McNemar test was used to assess efficacy of plating media and enrichment broths. A p-value of <0.05 denoted statistical significance.

3. Results

Of the 1096 stool samples processed by direct plating, a total of 102 stools were positive for *Salmonella* on at least one plating medium in which 87 (85.3%), 35 (34.3%), 30 (29.4%), and 35 (34.3%) were isolated from MSRV, MAC, HE, and XLD, respectively (**Figure 1**). A total 299/1297 stools samples were *Salmonella*-positive on at least one plating medium after enrichment procedure (**Figure 2**). The number of samples containing *Salmonella* spp. using RV, BPW, and SB Enrichment broths were 197 (86.0%), 180 (78.6%), and 177

(77.3%), respectively. All *Salmonella* isolates were nontyphi *Salmonella*.

Direct plating on MSRV yielded significantly more *Salmonella* isolates when compared to MAC, HE, and XLD (p-value < 0.0001) (**Table 1**). There was no statistical significance in numbers of *Salmonella*-positive stools when comparing MAC, HE and XLD. Enrichment with RV yielded significantly more *Salmonella* isolates when compared to BPW (p-value = 0.0405) and SB (p-value = 0.0126) (**Table 1**). Use of MSRV in combination with MAC and HE when compared to MAC + HE increased recoverability of *Salmonella* isolates by 3.9%, 55.5%, and 30.6% after enrichment cultures with RV, BPW, and SB respectively (**Table 2**).

4. Discussion

Improving isolation of *Salmonella* from human stool samples by using MSRV medium in clinical laboratories

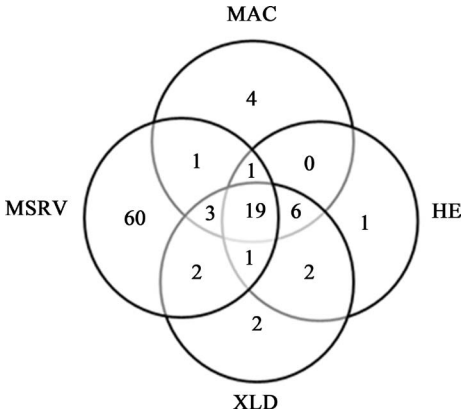

Figure 1. Venn diagram showing numbers of samples containing *Salmonella* on MAC, HE, XLD, and MSRV.

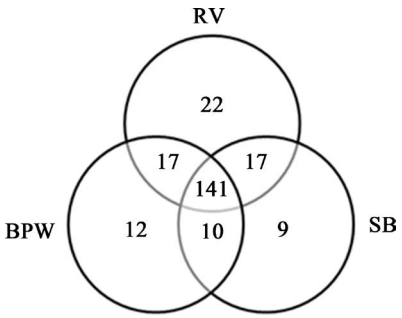

Figure 2. Venn diagram showing numbers of samples containing *Salmonella* recovered from RV, BPW, and SB enrichment broths.

Table 1. Statistical difference, based on numbers of *Salmonella*-positive stools, compared between four plating media and three enrichment broths.

Compared Media	Statistical Difference
	p-value
MAC vs. HE	0.3877
XLD vs. MAC	1.0000
MSRV vs. MAC	<0.0001
MSRV vs. HE	<0.0001
MSRV vs. XLD	<0.0001
XLD vs. HE	0.1797
RV vs. SB	0.0126
RV vs. BPW	0.0405
SB vs. BPW	0.7874

Table 2. Stool samples containing *Salmonella* compared among 4 selective agar media by direct plating and 3 enrichment broths.

Enrichment broths	Numbers (%) of *Salmonella*-positive stool samples recovered from		% Increase
	MAC + HE	MAC + HE + MSRV	
RV	188 (82.1)	197 (86.0)	3.9
BPW	53 (23.1)	180 (78.6)	55.5
SB	107 (46.7)	177 (77.3)	30.6

has been described previously. Aspinal *et al*. (1992) reported a 21.3% increase in isolates recovered by direct plating using MSRV compared to cultivable numbers utilizing selenite F broth enrichment prior to sub-culturing on brilliant green agar, while a 29% increase was indicated using Rappaport-Vassiliadis broth in conjunction with brilliant green agar. Furthermore, Dusch and Altwegg (1995) reported direct plating sensitivities of 63.4% for MSRV in contrast to 34.1% and 32.8% for HE and Xylose-Lysine-Tergitol 4 (XLT4) respectively [17]. Ruiz *et al*. (1996) reported the number of *Salmonella* isolates recovered by direct plating on MSRV was also greater than those obtained on SS, *Salmonella* identification detection agar medium (SM-ID), Rambach agar, novobiocin-brilliant green-glycerol-lactose (NBGL) agar, and selenite-SS combination [6]. More recently, Ruiz *et al.* (1998) found a clear performance of MSRV over SS by direct plating with 24.7% greater sensitivity. They did not obtain significant differences when MSRV was compared to the selenite-SS combination, but the sensitivity of the selenite-MSRV combination was 8.2% greater than that of selenite-SS combination (p-value < 0.01) [18].

Presently, we intended to compare the yields of *Salmonella* from MSRV to MAC, HE and XLD only by DP method because it is known that number of *Salmonella* contained in stool samples to prior enrichment is much lower than after enrichment and is suitable for comparison. The performance of MSRV medium provided a 51.0% increase in recoverability of non-typhoid *Salmonella* spp., when compared to MAC and XLD, while a 59.9% increase when compared to HE agar. Although RV broth yielded the highest number of *Salmonella*-positive samples, there are several disadvantages of the medium as it is not suitable for detection of non-motile strains of *Salmonella*, *S*. Typhi and *S*. Paratyphi A [16] [19]. The yield obtained from BPW was highest when MSRV was used in conjunction with MAC and HE. In order to isolate all *Salmonella* serotypes, BPW should be used for enrichment followed by using MSRV in combination with MAC, HE. An additional advantage of BPW is that it enhances the growth of other enteric bacteria pathogens to include *Shigella*, *Aeromonas*, *Plesiomonas shigelloides*, and the diarrheagenic *Escherichia coli*. For these reasons, our laboratory has instituted using MSRV medium in direct plating and in combination with our standard media after enrichment culture in BPW for the improvement of isolating *Salmonella* spp. from human stool samples.

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