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Comparative Study of Enteric Media in the Isolation of Salmonella And Shigella from Stool Specimens.

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Various media have been developed for isolation of gram-negative enteric pathogens from stool specimens. The isolation traditionally uses the combination of MacConkey agar as a differential plating medium, SS agar as a selective plating medium, and Selenite-F broth as an enrichment broth. Recent evidence has shown that MacConkey agar lacks selectivity for Salmonella and Shigella so lactose fermenters can overgrow or inhibit these organisms (1), whereas SS agar and Selenites-F broth have been to inhibitory for fastidious pathogens such as some species of Shigella (2,3,4). Furthermore, many of these media offer little in the way of colonial morphologic differentiation other than the ability of some organisms to ferment lactose.

In 1965, Taylor (5) introduced XLD agar of which the latter studies shown better rate of isolation of enteric pathogens which fewer false-positive organisms (3,4,6,7,8). On this medium the differentiation of Salmonella and Shigella from other enteric bacilli is based on not only the fermentation of lactose, saccharose, or xylose, but also on lysine decarboxylation and H_2S production.

More recent study of GN broth (3,4,6) have clearly demonstrated in the enhancement of isolation of enteric pathogens. In this paper routine stool specimens have been used to compare Direct Streaking : MacConkey, SS and XLD agar with Enrichment Broths : Selenite-F and GN broths for the isolation of Salmonella and Shigella.

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Meterials and Methods

Between 11 March and 5 June 1975, a total of 252 stool specimens from patients seen at Chiang Mai Hospital was examined for Salmonella and Shigella. Each stool specimen was streaked directly onto the three plates: MacConkey Agar (MC, Eiken), Salmonella Shigella Agar (SS, Eiken) and Xylose Lysine Deoxycholate Agar (XLD, Eiken) and also placed into the enrichment broths: Selenite Broth (SF, Eiken) and Gram Negative Broth (GN, BBL). After 18–24 hr. of incubation, the broths were streaked on MC and XLD plates. Colonies suspected of being Salmonella and Shigella were picked and inoculated onto Triple sugar iron agar (TSI) slants. Following overnight incubation, TSI slants showed the reaction: alkaline slants, acid butt with or without H_2S were inoculated into motility - indole, Simmon's citrate, urea, lysine decarboxylase and phenylalanine medium. Cultures showing biochemical reactions for Salmonella and Shigella were tested agglutinably with specific antisera for Salmonella and Shigella (Difco) respectively.

Results

Of the 252 stool specimens examined, 59 (23.4%) were positive for the enteric pathogens; 37 (14.7%) were Shigella and 22 (8.7%) were Salmonella (Table 1). Although all three media used for isolation of Shigella by direct streaking were approximately equal, no one medium grew all of them. Of the 37 isolates of Shigella, all three media used in parallel accounted for 36 (97.3%), whereas one kind of media detected no more than 28 (75.7%).

Comparisons between direct streaking and enrichment methods for isolation of enteric pathogens are shown in Table 2. Selenite enrichment streaked to XLD plate was observed to be greatly superior to both direct streaking and other enrichment methods for detection of Salmonella. Of all Salmonella isolated only 9 (40.9%) isolates were obtained from direct streaking, whereas S F broth streaked to XLD recovered 19 (86.5%). However, all of them were isolated by the use of both direct streaking and SF enrichment streaked to XLD agar. In contrast, direct streaking

Table 1. Isolation of Salmonella and Shigella from 252 Stool Specimens.

Organisms	Direct Streaking				Enrichment Broths					Total	
					GN		SF				
	MC	SS	XLD	Sub-total	MC	XLD	MC	XLD	Sub-total	No.	Percent
Salmonella No.	1	7	5	9	1	3	6	19	19	22	8.7
Salmonella percent	4.5	31.8	22.7	40.9	4.5	13.6	27.3	86.4	86.4	100	
Shigella No.	28	26	25	36	5	8	2	2	9	37	14.7
Shigella percent	75.7	70.3	67.6	97.3	13.5	21.6	5.4	5.4	24.3	100	
Total	29	33	30	45	6	11	8	21	28	59	23.4

was seen to be clearly superior to enrichment for isolation of Shigella. Of the total Shigella isolated, direct streaking accounted for 36/37 (97.3%) whereas enrichment broth detected only 9/37 (24.5%).

The distribution of 22 isolates of Salmonella was shown in Table 3. Of these, 14 (63.6%) were Salmonella serogroup B of which 11 isolates were recovered from XLD inoculated from SF broth. The species of Shigella are shown in Table 4. Shigella flexneri was the most frequently isolated species: 78.4% of the total Shigella isolated and all of them can be found by direct streaking.

Table 2 Comparison of Direct Streaking and Enrichment Broth Methods for Isolation of 22 Isolates of Salmonella and 37 isolates of Shigella.

Organisms	Direct Streak only*	Enrichment only				Both Methods	Total
		GN		SF			
		MC	XLD	MC	XLD		
Salmonella	9	1				0	10
	8		2			1	11
	7			4		2	13
	3				13	6	22
Shigella	31	0				5	36
	29		1			7	37
	35			1		1	37
	35				1	1	37

*Number of organisms isolated from direct streaking method corresponds with one of the four enrichment methods.

Table 3 Distribution of Salmonella isolated from 22 Positive Stool Specimens

Organisms	Direct Streaking				Enrichment Broths					Total	
	MC	SS	XLD	Sub-total	GN MC	GN XLD	SF MC	SF XLD	Sub-total	No.	Percent
Salmonella Serogroup B	1	6	4	8	0	3	3	11	11	14	63.6
Salmonella typhi	0	1	1	1	1	0	2	4	4	4	18.2
Salmonella serogroup E	0	0	0	0	0	0	1	4	4	4	18.2
Total	1	7	5	9	1	3	6	19	19	22	100.0

Table 4 Distribution of Shigella isolated from 37 Positive Stool Specimens

Organisms	Direct Streaking				Enrichment Broths					Total	
	MC	SS	XLD	Sub-total	GN MC	GN XLD	SF MC	SF XLD	Sub-total	No.	Percent
S. dysenteriae	0	1	0	1	0	1	1	1	1	2	5.4
S. flexneri	24	20	22	29	4	5	0	0	6	29	78.4
S. boydii	2	1	1	2	1	1	1	1	1	2	5.4
S. sonnei	2	4	2	4	0	1	0	0	1	4	10.8
Total	28	26	25	36	5	8	2	2	9	37	100.0

Discussion

The efficacies of MacConkey, SS and XLD agars were similar for Isolation of Shigella but for Salmonella there were fewer isolates from MC than from either SS or XLD agar when the stool specimens were plated directly. In isolation of both organisms, however, XLD agar was superior to MC when the specimens were plated indirectly with enrichment broth. This result is similar to that reported by Taylor and Schelhart (7) and Morris et. al. (9). Results with combination of media showed that only the combination of direct streaking and enrichment in SF

broth streaked to XLD medium can detect all 59 isolates of enteric pathogens.

Of all pathogens isolated, Shigella flexneri was the most frequently isolated species (49.1%). These organisms were all isolated by direct streaking. In isolation of all Shigella, direct streaking was markedly superior to GN and SF enrichment. This observation agreed with some reports (1, 3, 9, 11, 12) but others had shown the greater isolation of Shigella by the use of enrichment broth (4, 6, 7, 10, 13). In addition, no more than 75.7% of the Shigella were obtained from one kind of plates, whereas all three memia used in parallel increased up to 97.3% of isolates.

In contrast to the Shigella data, Selenite enrichment was observed to be greatly superior to direct streaking for detection of Salmonella. Besides, SF broth streaked to XLD was the best enrichment method. This was similar to that reported by Taylor and his colleagues (6, 7, 10, 11).

The results of this study indicate that, in the examination of a stool specimen suspected of harboring either Salmonella or Shigella, it would be desirable to plate the specimen directly on MC, SS and XLD agar and also to inoculate the same specimen in Selenite broth for enrichment before plating on XLD agar.

ย่อเรื่อง

การศึกษาเปรียบเทียบระหว่างอาหารเลี้ยงเชื้อที่ใช้แยกเชื้อ Salmonella และ Shigella จากอุจจาระของผู้ป่วยที่มารับการรักษาที่โรงพยาบาลนครเชียงใหม่ จำนวน 252 ราย อุจจาระแต่ละรายนำไปตรวจหาโดยใช้วิธี Direct Streaking คือเลี้ยงบน MacConkey Agar SS Agar และ XLD Agar และโดยวิธี Enrichment Broth คือเลี้ยงใน GN Broth และ Selenite Broth แล้วจึง นำไปเลี้ยงต่อบน MacConkey และ XLD จากวิธีดังกล่าวสามารถแยกเชื้อ Enteric pathogens ได้ทั้งหมด 59 isolates ซึ่งในจำนวนนี้แยกเป็น Shigella เสีย 37 isolates และ Salmonella 22 isolates นอกจากนี้ยังพบเชื้อ Shigella flexneri มากที่สุดคือ พบถึง 49.1%

จากการแยกเชื้อ Shigella พบว่าวิธี Direct Streaking ให้ผลดีกว่าวิธี Enrichment Broth ไม่ว่าจะใช้วิธีใด คือ วิธี Direct Streaking บนอาหารเลี้ยงเชื้อทั้ง 3 ชนิด

สามารถพบเชื้อได้ถึง 97.3 % ขณะที่วิธี Enrichment Broth ตรวจพบเพียง 24.3 % เมื่อเปรียบเทียบอาหารเลี้ยงเชื้อทั้ง 3 ชนิดที่ใช้ในวิธี Direct Streaking พบว่าแต่ละชนิดมีประสิทธิภาพพอๆ กัน และสามารถแยกเชื้อได้ไม่เกิน 75.7%

สำหรับการตรวจแยกเชื้อ Salmonella พบว่าให้ผลตรงข้ามคือ เมื่อใช้วิธี Selenite Enrichment Broth แล้วนำไปเลี้ยงบน XLD Agar สามารถตรวจพบเชื้อได้สูงถึง 86.4% ขณะที่วิธี Direct Streaking ให้ผลเพียง 40.9 % แต่ถ้าใช้ทั้งสองวิธีดังกล่าวร่วมกันก็จะให้ผลการแยกเชื้อดีที่สุด จากผลการศึกษานี้แสดงให้เห็นว่าการตรวจแยกเชื้อทั้ง Salmonella และ Shigella จากอุจจาระในเวลาเดียวกัน ควรใช้วิธี Direct Streaking บน MacConkey, SS และ XLD Agars ร่วมกับวิธี Selenite Enrichment Broth ที่ต้องนำไปเลี้ยงต่อบน XLD Agar.

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