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ISOLATION OF ENTERIC PATHOGENS *

"Comparison of different enrichment and plating media for recovery of medically important bacteria from human stool specimens"

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ABSTRACT

To isolate enteropathogenic bacilli from patients' stool, One gram samples of fecal specimens were homogenized using glass bead, and swabs were saturated from this suspension and used to inoculate the various agar media and different enrichment broth media. After over night incubation Mc. and SS. agar were inoculated from the enrichment broth media.

Over a one year period, stool cultures of clinical diarrhoea cases yielded 16 strains of Salmonella typhi, 2 Shigella dysenteriae, 12 Shigella flexneri, 4 Shigella sonnei, and 11 Proteus morganii. No media proved specific for isolation of pathogenic bacteria.

It was the purpose of this investigation to study isolation of enteropathogenic bacilli causing diarrhoea in patients, comparing different enrichment and plating media, and determining susceptibility of enteric bacilli to antibiotics.

MATERIALS AND METHODS

To isolate enteropathogenic bacilli (Salmonella, Shigella, Pathogenic E. coli, V. cholera, and Arizona,) from Patients' one gram samples of fecal specimens were homogenized using glass beads, and swabs were saturated from this suspension and used to inoculate the various media (Mac Conkey agar (Mc), Eosin methylene blue agar (EMB), Xylose lysine medium(XLM), Salmonella and Shigella agar (SS), Brilliant green agar (BG), and Bismuth sulfite agar

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(BS) and different enrichment media (Heart infusion broht (HIB), Gram negative broth (GNB), Selenite broth (BS), and Tetrathionate broth (TB). After overnight incubation Mc. and SS. agar were inoculated from the enrichment media.

Identification of enteropathogenic bacilli was by appropriate biochemical tests and serological identification. Susceptibility of enteropathogenic bacilli to antibiotics (Chloramphenicol, Erythomycin, Streptomycin, Kanamycin, and Tetracycline) was determined on the pure culture isolates, using a standardized diffusion technique.

Known cultures (Salmonella species, Shigella species, pathogenic **E. coli Proteus morganii**, and **Vibrio cholera** species) were studies by culturing in Heart infusion broth at 37° C overnight, and then diluting the culture with HIB. to 1 : 1000, (0.05 ml broth culture diluted to 50 ml with HIB). A swab was used to inoculate each diluted culture onto Mc., XLM., EMB., SS., BG., and BS.,. Four swabs immersed into the same dilute culture were then put into tubes of HIB., GNB., SB., and TB. plates and tubes were incubated at 37° C overnight. plats were then examined and enrichment broth cultures subcultured to Mc. and SS., which were then examined after 37° C overnight incubation.

Known mixed cultures were studied by using two diluted cultures (1 : 1000) mixed together in the ratio 1 : 1. Diluted mixed cultures were inoculated onto the plates and in broth uaing the same method as in the known cultures study.

RESULTS

Salmonella species isolated from clinical specimens included three **Salmonella typhi** (S) from stools, three **Salmonella typhi** (B) from blood, one **Salmonella typhi** (U) from urine and **Salmonella para** C_1. All Salmonella species grew well on the plates except BS., on which some strains were inhibited; in broth cultures, all of the organisms grew well (table I.).

Of Shigella species cultivated on Mc., EMB., XLM., SS., BG., and BS., agar plates, most were inhibited on SS. and BS. agar plates. Some Shigella species were inhibited in SB. (table II)

Escherichia coli 0119 : B 14 grew on Mc., EMB., XLM., BG., BS., but it was inhibited on SS. agar. In HIB., GN., SB., TB. cultures, it grew well. **Proteus Morganii** grew on Mc., EMB., SS., BG., but did not grew on BS. agar. In all broth cultures, it grew well (table III).

11 **Vibrio cholera Eltor** (Inaba phage type 8) were slightly inhibited on EMB., SS. and completely inhibited on BS. agar. All grew well in HIB., GN., SB. and TB. (table IV)

Of the cultures of pathogenic Bacilli mixed (1:1) with **E. coli** from human stools, some were overgrown by **E. coli.**

(pathogenic bacilli could not be isolated) (table V)

Over a one year period, stool cultures of clinical diarrhoea cases yielded 16 strains of **Salmonella typhi**, 2 **Shigella dysenteriae**, 12 **Shigella flexneri**, 4 **Shigella sonnei** and 11 **Proteus morganii.** No media proved specific for pathogenic enteric bacteria (table VI)

Results of antibiotic sensitivity tests (Chloramphenical, Erythromycin, Streptomycin, Kanamycin, and Tetracycline) showed most Salmonella typhi sensitived to Chloramphenicol. Other organisms varied (table VII)

CONCLUSION

Taylor and Harris (1965) compared different enrichment (TSB., GNB., Siliker, SF., TT.) and plating media (EMB., Mc., XLM.) for culture of Shigella species. Media found suitable for Shigella species were EMB., XLM., Mc., TSB., GNB and Siliker broth.

Gerichter and Sechter (1966) isolated Salmonella species from bone meal, and found BS. better than SS. agar.

In this study, comparison of different enrichment media and plating media for recovery of medically important bacteria from human stool specimens showed that most Salmonella species could be isolated from SS., BG., and BS agar. Other enteropathgenic bacilli were most often isolated from Mc., EMB. and XLM. If direct plating was positive, the enrichment culture was positive, but if direct plating was negative, the enrichment culture was negative too.

In routine work, isolation of enteric bacilli must combine strongly inhibitory media (SS., BS., BG.) with less inhibitory media (Mc., EMB., XLM.) for maximum recovery of pathogenic bacteria,

Table. I Cultivation of Salmonella species.

Organisms	Direct culture on						culture in Broth			
	Mc	EMB	XLM	SS	BG	BS	HIB	GN	SB	TB
S. typhi S 1	pn	n	pn	pn	pn	ps	+ve	+ve	+ce	+ve
S. typhi S 2	pn	ps	pn	pn	pn	ps	+ve	+ve	+ve	+ve
S. typhi S 3	pn	pn	pn	pn	pn	ps	+ve	+ve	+ve	+ve
S. typhi B 1	pn	ps	pn	pn	pn	n	+ve	+ve	+ve	+ve
S. tyyhi B 2	pn	pn	pn	pn	pn	ps	+ve	+ve	+ve	+ve
S. typhi B 3	pn	pn	pn	pn	pn	ps	+ve	+ve	+ve	+ve
S. typhi U	pn	pn	pn	pn	pn	ps	+ve	+ve	+ve	+ve
S. typhi CI	pn	ps	pn	pn	pn	ps	+ve	+ve	+ve	+ve
S. typhi CI	pn	pn	pn	pn	pn	n	+ve	+ve	+ve	+ve

Table. II Cultivation of Shigella species.

Organisms	Direct culture on						Culture in Broth			
	Mc	EMB	XLM	SS	BG	BS	HIB	GN	SB	TB
Sh. dysentery 1	pn	pn	pn	n	ps	n	+ve	+ve	n	+ve
Sh. dysentery 2	pn	ps	pn	pn	pb	n	+ve	+ve	+ve	+ve
Sh. flexneri 1	pn	pn	pn	ps	pn	n	+ve	+ve	n	+ve
Sh. flexneri 2	pn	pn	pn	ps	pn	n	+ve	+ve	n	+ve
Sh. sonnei 1	pb	pn	ps	n	pb	n	+ve	+ve	+ve	+ve
Sh. sonnei 2	pb	pn	pn	n	ps	n	+ve	+ve	n	+ve
Sh. boydii 1	pb	pn	pn	n	pn	n	+ve	+ve	+ve	+ve
Sh. boydii 2	pn	pn	pn	n	pb	pn	+ve	+ve	+ve	+ve
alkalarescens Dispar.	pn	pn	pn	pn	pb	n	+ve	+ve	+ve	+ve

pn= positive normal size, ps= positive small size,

pb= positive big size, n= no growth.

Table. III Cultivation of **E. coli** and **Proteus morganii.**

Organisms	Direct culture on						Culture in Broth			
	Mc	EMB	XLM	SS	BG	BS	HIB	GN	SB	TB
E.Coli 0019:B14	pn	ps	pn	n	pn	pn	+ve	+ve	+ve	+ve
Prot. morganii	pn	ps	pn	pn	pn	n	+ve	+ve	+ve	+ve

Table. IV Cultivation of **Vibrio cholera Eltor** (Inaba phage type 8)

Organisms	Direct culture on							Culture in Broth			
	Mc	EMB	XLM	SS	BG	BS	TA*	HIB	GN	SB	TB
Vicholera 2	pn	ps	pn	ps	pb	n	pn	+ve	+ve	+ve	+ve
,, 3	pn	ps	pn	ps	pb	n	pn	+ve	+ve	+ve	+ve
,, 4	pn	ps	pn	ps	pb	n	pn	+ve	+ve	+ve	+ve
,, 7	ps	ps	pn	ps	pb	n	pn	+ve	+ve	+ve	+ve
,, 10	pn	ps	pn	ps	pb	n	pn	+ve	+ve	+ve	+ve
,, 13	pn	ps	pn	ps	pb	n	pn	+ve	+ve	+ve	+ve
,, 14	pn	ps	pn	ps	pn	n	pn	+ve	+ve	+ve	+ve
,, 15	pn	ps	pn	pb	pb	n	pn	+ve	+ve	+ve	+ve
,, 16	pn	ps	pn	ps	pn	n	pn	+ve	+ve	+ve	+ve
,, 17	pn	ps	pn	ps	pn	n	pn	+ve	+ve	+ve	+ve
,, 18	pn	ps	pn	ps	pn	n	pn	+ve	+ve	+ve	+ve

* TA = Tellurite Agar for V. cholera (SEATO Laboratory)

Table. V Cultivation of pathogenic bacilli mixed with E. coli from human stools.

Organisms	Direct culture on						Culture in Broth			
	Mc	EMB	XLM	SS	BG	BS	HIB	GN	SB	TB
E. coli 0119 : B 14+S. typhi	E	E	E	S	E	E	E	E	S	E
E. coli+S. typhi	S	S	S	S	S	S	E	E	E	E
E. coli+Sh. sonnei	Sh	Sh	Sh	E	Sh	Sh	Sh	Sh	E	Sh
E. coli+S. typhi	S	S	S	S	S	E	S	S	S	E
E. coli+Sh. boydii	Sh	Sh	Sh	E	Sh	E	E	E	Sh	E
E. coli+Sh. flexneri	Sh	Sh	Sh	Sh	Sh	E	Sh	Sh	Sh	Sh
E. coli+S. para C	S	S	S	S	S	S	S	S	S	S

Table VI. Isolution of enteropathogenic bacteria from patients.

Organisms	Direct culture on						Culture in Broth			
	Mc	EMB	XLM	SS	BG	BS	HIB	GNB	SB	TB
16, Sal. typhi	12	12	11	15	8	9	14	15	14	15
2, Shig. dysentery	1	1	1	1	1	0	0	1	1	1
12, Shig. flexneri	9	8	6	9	6	1	8	9	6	3
4, Shig. sonnei	4	3	3	2	2	4	4	3	4	2
11, Prot. morganii	6	5	4	5	3	0	5	8	8	4

Table VII. Susceptibility of enteropathogenic bacteria to antibiotics.

Organisms	Chloram			Erythro.			Strepto.			Kana			Tetra.		
	S	In	R	S	In	R	S	In	R	S	In	R	S	In	R
16, Sal. typhi	14	2	0	0	1	15	1	1	14	11	5	0	14	2	0
2, Shig. dysentery	1	0	1	0	0	2	1	0	1	1	0	1	1	0	1
12, Shig. flexner	4	0	8	0	1	11	0	2	10	4	6	2	4	1	7
4, Shig. sonnei	2	0	2	0	0	4	0	1	3	2	2	0	2	1	1
11, Prot. morganii	0	1	10	0	0	11	0	1	10	1	5	5	0	1	10

S = Sensitive, In = Intermediate, R = Resistant.

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