THE APPLICABILITY OF CARY BLAIR MEDIUM AS A SOLE TRANSPORT MEDIUM FOR THE RECOVERY OF ENTEROPATHOGENS IN THE MALAYSIAN CONTEXT

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SUMMARY

The efficacy of Cary Blair medium was compared with that of Selenite 'F' in the isolation of enteropathogens from 805 cases of diarrhoea. It was shown that use of the Cary Blair medium resulted in a significantly higher rate of isolation. Not only were organisms found which were not isolated from the Selenite 'F' but there was a much higher isolation rate of Salmonella from Cary Blair medium althougn Selenite 'F' is generally considered as an enrichment medium for Salmonella. The findings indicate that it would be advantageous to introduce Cary Blair medium as the sole transport medium for the isolation of enteropathogens from cases of diarrhoea.

INTRODUCTION

The ideal specimen for bacteriological diagnosis of diarrhoea is faeces freshly collected and processed in the laboratory with minimal delay. It may be possible to achieve this in a laboratory situated within and serving its own hospital. However this does not hold true for the majority of diarrhoea cases in this country where the time lapse between collection of specimen and the processing of it in the laboratory can range from one to six days depending on the distance to be travelled and the "efficiency" of the transport logistics. Under these circumstances fresh stools or rectal swabs will be useless as the pathogens may have died or have been masked by rapidly growing commensal organisms. It has therefore been a long standing practice at the bacteriology division, Institute for Medical Research and many of the state pathology laboratories to receive stool specimens or rectal swabs in Selenite 'F' and or alkaline peptone enrichment media. The choice of these media was based on the desire to isolate *Salmonella* and *Vibrio cholerae* which were considered to be the most important enteropathogenic bacteria. However these two media are not suitable for the isolation of other enteropathogens such as enterotoxigenic *E.coli* and *Shigella* and consequently the isolation rates of these other enteropathogens has been low in laboratories depending solely on these transport media for faecal specimens. However, in order to fully appraise the role played by these other enteropathogens in the causation of diarrhoea in this country there is a need to use a transport mechanism which does not preclude their isolation.

A transport medium was developed by Cary and Blair (1964) for the collection and transport of clinical specimens. Their preliminary studies on faecal specimens showed that *Salmonella* and *Shigella* could be recovered for as long as 49 days and *Vibrio cholerae* for 22 days. A subsequent field

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trial conducted with this medium (Gaines *et al*, 1965) showed its efficacy in isolating *Salmonella, Shigella,* enteropathogenic *E.coli* and *Vibrio cholerae*. The efficacy of this medium for isolation of *Vibrio cholerae* was further attested by De Witt *et al* (1971) and for *Vibrio parahemolyticus* by Neumann *et al* (1972).

It was felt therefore that we should introduce the Cary Blair medium as the sole transport medium in this laboratory. Before this could be done however, we wished to show that use of this medium at the expense of Selenite 'F' did not result in us missing some *Salmonella* isolates. A study was therefore designed to compare the use of Cary Blair medium and Selenite 'F' for screening specimens from diarrhoea cases.

MATERIALS AND METHODS

Cary Blair media, Selenite 'F' media and cotton swabs were supplied to private and government hospitals and clinics with specific instructions on how to use them. Two swabs were to be used for each case, one to be dipped into the Selenite 'F' and one into the Cary Blair medium. The district hospitals were situated in Bentong, Kajang, Kelang, Kuala Kubu Baru, Kuala Lipis, Mentakab and Sungei Buloh while the private clinics were all situated in Kuala Lumpur. The study period was from January 1977 to March 1978. Specimens from Kuala Lumpur were usually processed within a day of collection while it took between two to six days for specimens from other areas to reach the laboratory.

On receipt at the laboratory, the Selenite 'F' media were incubated overnight before further processing while the swabs from the Cary Blair media were inoculated on to MacConkey agar, desoxycholate citrate agar (DCA), selenite 'F' broth, alkaline peptone water and in cases of children below 10 years of age into Robertson's cooked meat media containing 10 percent salt and all of these were incubated at 37°C. After overnight incubation the broth cultures were inoculated onto appropriate solid media, namely MacConkey and DCA for the Selenite 'F', Monsur's agar for the alkaline peptone water and blood agar for the Robertson's cooked meat medium and incubated overnight.

TABLE I
COMPARISON OF ISOLATION OF 'ENTEROPATHOGENS' FROM SELENITE 'F' AND CARY-BLAIR TRANSPORT MEDIUM FROM THE SAME PATIENTS

"Enteropathogen"	Selenite 'F'	Cary-Blair
Salmonella	16	23
Shigella flexner	-	11
Shigella sonnei	3	6
Shigella dysenteriae	-	1
Enteropathogenic *E.coli*	-	15
V.parahemolyticus	-	1
Staphylococcus aureus	-	18
Total	19	75
No. of specimens examined	805	805
Isolation rate	2.4%	9.3%

TABLE II
DISTRIBUTION OF *SALMONELLA* SEROTYPES ISOLATED FROM SELENITE 'F' AND CARY-BLAIR MEDIA

Serotype	No of isolations from	
	Selenite 'F'	Cary-Blair
S.haifa	1	1
S.paratyphi B	1	3
S.typhimurium	2	2
S.derby	1	1
S.stanley	1	2
A.emek	1	-
S.singapore	-	1
S.meleagridis	1	1
S.weltevreden	3	5
S.london	-	1
S.lexington	1	2
S.bovis morbificans	2	2
S.javiana	1	1
S.untypable	1	1
Total	16	23

Suspicious looking colonies on all the plates were inoculated on to triple sugar iron (TSI) slopes. Colonies showing characteristic reactions on TSI slopes were further processed for identification

according to recommended procedures. (Edward and Ewing, 1972)

Faecal samples from 805 cases were received in Cary-Blair medium and Selenite 'F'. Table I compares the isolations from both of these media.

The increased isolation rate from the Cary Blair transport medium is easily apparent. There was an overall isolation rate of 9.3 percent as compared to 2.4 percent from the Selenite 'F' medium. 23 *Salmonella* strains were isolated as compared to only 16 from the Selenite 'F'. There was only one *Salmonella* serotype (*S.emek)* which was found in the Selenite but not picked up from the Cary Blair. Table II lists the *Salmonella* isolated from the two media.

Shigella flexneri was only isolated from Cary Blair medium; 3 isolates of *Shigella sonnei* were found in the Selenite while these and three others were isolated from Cary Blair. One strain of *Shigella dysenteriae,* 15 of enteropathogenic *E.coli,* 1 of *Vibrio parahemolyticus* and 18 of *Staphylococcus aureus* were found only in Cary Blair media. Table III lists the serotypes of enteropathogenic *E.coli* isolated.

TABLE III
SEROTYPES OF ENTEROPATHOGENIC *E.COLI* ISOLATED FROM CARY BLAIR MEDIUM

Serotype	Number isolated
055 :K59	2
0111:K58	1
0114:K90	1
0119:K69	1
0125:K79	2
0127:K63	1
0128:K67	2
0142:K86	5
Total	15

DISCUSSION

The study has shown that use of the Cary Blair medium resulted in a higher rate of isolation of enteropathogens than Selenite 'F' medium. Not only were organisms found which were not at all isolated from Selenite 'F' such as enteropathogenic *E.coli, V.parahemolyticus* and *Staphylococcus aureus* but there were increased isolations of *Salmonella.* This was a surprise finding as Selenite 'F' is presumed to be a selective and enrichment medium of *Salmonella* and one would have expected to get an isolation rate at least the same, if not better than that obtained from Cary Blair medium. This shows that there is very little justification for the use of Selenite 'F' medium as the sole transport medium in the investigation of cases of diarrhoea. The findings of this study indicate that if only one medium is to be used Cary Blair transport medium would be a better choice than Selenite 'F' medium.

There is a crying need for a single transport medium for the despatch of stool specimens from areas some distance from the laboratory. Use of a multiplicity of media may improve the range and numbers of different enteropathogens isolated but it adds on to cost and labour and very often instructions are not followed accurately. It is not unusual for some media in the set to be returned with others left out either due to a lack of understanding on the part of the specimen collector or simply because one or other of the media has been depleted. These were the difficulties which slowly led to the evolution of the use of Selenite 'F' as the single transport/ enrichment medium. The present study has confirmed the fallacy of this and recommendations will be made to introduce Cary Blair medium as the sole transport medium for stool specimens where it will be impractical to expect fresh stools.

The use of Cary Blair medium is not without its pitfalls. Some people in the field are generally resistant to change and will be uncomfortable when a hitherto well used procedure is suddenly abandoned. Furthermore, to be effective, the swab has to be dipped into the Cary Blair and not merely deposited on the surface. Our experience has shown that very often this is ignored although specific written instructions have accompanied the Cary Blair bottles.

Although we did not have an opportunity to prove the efficacy of Cary Blair medium in the investigation of cholera cases and carriers as one would have to wait for an outbreak to test this out, other studies (DeWitt *et al* 1971) have shown that it can be depended upon. In view of this it appears reasonable that it may not be necessary to include collection of specimens in alkaline peptone water provided Cary Blair is used.

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