The Bacterial Causes of Diarrhoea in Malaysia

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DIARRHOEAL DISEASES are the cause of much morbidity and mortality in developing countries, particularly in the very young. In areas where health conditions are poor, acute gastro-intestinal infections account for a high proportion of deaths.

An idea of the problem in Peninsular Malaysia is obtained when returns of gastro-enteritis cases from government health facilities are examined (1). In 1972 there were altogether 123,422 cases of gastro-enteritis reported to the epidemiology unit at the Ministry of Health, Malaysia while in 1973 there was 131,552.

In this region, even in an urban society like Singapore, acute gastro-enteritis is the commonest condition seen in hospital paediatric practice (2).

Many agents have been held responsible for the causation of diarrhoea. Infective diarrhoea is caused by bacteria, parasites and viruses. Other causes of diarrhoea include the malabsorption syndromes, cystic fibrosis, gluten induced enteropathy, disaccharide intolerance, metabolic diseases, feeding mismanagement, parenteral infection, intolerance to drugs, milk allergy and surgical conditions (3).

Acute diarrhoeal disease is believed to have its basis in bacterial infections despite the observation that specific enteric pathogens may be isolated from only a relatively small percentage of individuals affected with diarrhoea (4).

In spite of this relatively small yield of enteric pathogens from clinical specimens it is necessary to carry out investigations of the etiology of diarrhoea for the purpose of epidemiologic studies as well as for the institution of the appropriate lines of treatment and clinical management.

In the present study, faeces from 3,809 patients (of all ages) with diarrhoea as the presenting symptom were investigated for the presence of recognised enteropathogenic bacteria. The purpose of the study was to see in what percentage of these cases could be recognised bacterial enteropathogen be isolated; the relative prevalence of the different bacteria found; and to see if there were any significant differences among the different age and racial groups and between males and females.

Materials and Methods

Specimens of faeces from cases of diarrhoea were sent to the bacteriology division, Institute for Medical Research, Kuala Lumpur. The study period commenced in September 1972 and was completed in August 1973. Specimens were sent from various parts of Malaysia including the towns of Seremban, Tampin, Bahau, Jelebu, Kuala Pilah, Port Dickson, Kajang, Pekan, Dungun, Kemaman, Kota Bharu, Kuala Lipis, Kuala Kubu Bharu, Kuantan, Mentakab, Bentong, Kuala Trengganu, Temerloh and the paediatric wards of the General Hospital, Kuala Lumpur. These contained a mixture of cases from both urban and rural areas.

Either fresh stool was sent (in cases where transport time was minimal) or stools were sent in Selenite medium, glycerine phosphate holding medium and alkaline peptone water. Specimens arriving in transport and selective media were incubated overnight before being subcultured on to DCA and MacConkey and Blood Agar plates and on to Monsur's medium in the case of alkaline peptone water specimens. Fresh faeces were plated directly on to the above media and also into selenite F. which was subcultured after overnight incubation. Further identification was based on biochemical reactions and agglutination with polyvalent and specific antisera.

Salmonella, Shigella, Staphylococcus aureus, Vibrio cholerae, Vibrio parahaemolyticus and Plesiomonas shigelloides were looked for.

Enteropathogenic E. coli and Staphylococcus aureus were specifically looked for only in children under 10 years of age. For the isolation of Staphylococcus aureus specimens were inoculated into Robertson's cooked meat medium with 10% salt.

For purposes of discussion the cases were classified according to race, sex and the following age groups -0.1 week, 1 week to less than a year, 1 to 5 years, 6 to 10 years and older than 10 years.

During the study period there was an outbreak of cholera in Malaysia and specimens sent from these cases, suspects and contacts were sent only in alkaline peptone water and thus were not suitable for examination of other pathogens. Because an outbreak of this nature would not reflect the true distribution of enteropathogens it was decided not to include in the study specimens sent only in alkaline peptone water for the specific isolation of cholera vibrios from contacts and cases.

Results

Table I shows the distribution of cases studied and the isolation rates of bacterial enteropathogens for the different age and ethnic groups, while Table II lists out the various isolates including those from mixed infections.

Isolation rates:

3,809 specimens of faeces were studied. In 605 of these recognised enteropathogens were isolated. This gives an overall isolation rate of 15.9%.

The isolation rates can be considered separately for the different age groups. Two isolates came from patients whose age group was not known. From these figures it appears that isolation rates for bacterial pathogens are progressively lower as the age of the patient increases.

Application of the X^2 test shows these figures to be significant (p<0.05) in all cases except for comparison between the 0-1 week and less than a year groups, the less than 1 year and the 6-10 years groups, the 1-5 and the 6-10 age groups.

Similarly the isolation rates may be compared for the different racial groups. Application of X^2 test shows significant difference (p<0.05) when the Chinese are compared with the Malays, and Indians. The difference seen between the Malays and Indians is not statistically significant.

Pathogens isolated:

605 isolates were found of which Salmonella constituted 51.0% (309 isolates); Enteropathogenic E. coli, 30.6% (185); Shigella, 9.3% (56); Staphylococcus aureus, 4.9% (30); mixed infections, 3.5%(21); Vibrio parahemolyticus, 0.5% (3) and Plesiomonas shigelloides, 0.2% (1). There were no isolates of V. cholerae from these cases.

Salmonella infections:

Salmonella infections formed the largest group of pathogens isolated (51.0%).

Including the Salmonella which featured in mixed infections there were altogether 327 isolates composed of 22 different serotypes (Table II). S. typhimurium was by far the most prevalent accounting for 189 isolates. Others which were relatively frequent were S. weltevreden (28 isolates), S. typhi (18 isolates) and S. paratyphi B (14 isolates).

S. typhimurium

The percentage of individuals in which this organism was isolated is shown in Table III.

From these figures it appears that the younger age groups are more commonly affected and also that Chinese patients seem to have a higher incidence. The differences seen between the Chinese and the other groups is found to be statistically highly significant (p < 0.01). The difference seen between the Malays and Indians is also highly significant. When age groups are considered, the differences seen between those less than a year old and the older age groups is statistically significant.

Salmonella weltevreden:

The distribution pattern of isolates of S. weltevreden is seen in Table IV.

From the figures it appears that Indians and 'others' appear to be affected more than the other racial groups. However this difference was not found to be statistically significant. Similarly the higher incidence seen in the older age groups is not statistically significant except when the less than one year old group is compared with the older than 10 years age group.

Table I

Distribution of Cases Studied and Isolation Rates

AGE GROUPS

f		0 – 1 week	week	-	week -	1 week – 1 year		1 – 5 years	ears		6 - 10 years	rears		>10 years	ears	Total	Teolotion
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Malays	11	15	26	251	170	421	121	77	198	37 31	31	68	406	406 240	646	1359	13.5%
Chinese	34	31	65	300	190	490	131	55	186	22	26	48	231	192	423	1212	21.2%
Indians	S	11	16	191	191 160	351	105	83	188	31	22	53	211	200	411	1019	14.0%
Others	0	0	0	13	2	20	13	8	21	11	11 7	18	87	73	160	219	9.1%
Total	50	57	107	755	755 527	1282	370 223	223	593	101	86	187	935	705	1640	3809	15.9%
Isolation rates			31.8%			23.8%			19.2%			18.7%			7.1%		

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Table II DISTRIBUTION OF ISOLATES (this includes isolates from mixed infections)

			Racial groups		
Age group	Malays	Chinese	Indians	Others	Overal
0 – 1 week	19.2%	6.2%	12.8%	0%	10.3%
< 1 year	5.7%	14.3%	6.5%	10%	9.3%
1 - 5 years	0.5%	8.0%	8.0%	0%	5.3%
6 - 10 years	0%	8.3%	3.8%	5.6%	3.7%
> 10 years	0.4%	2.6%	1.7%	0.6%	1.3%
overall	2.4%	8.6%	4.6%	1.8%	

 Table III

 Distribution of S. typhimurium isolates

Table IV

Distribution of S. weltevreden isolates

			Racial groups		
Age group	Malays	Chinese	Indians	Others	Overal
0 - 1/52	0%	0%	0%	0%	0%
< 1 year	0%	0.6%	0.3%	5.0%	0.4%
1 - 5 years	0%	0%	1.1%	0%	0.3%
6 - 10 years	0%	0%	3.8%	0%	1.1%
> 10 years	1.2%	0.7%	1.5%	1.3%	1.2%
overall	0.6%	0.5%	1.1%	1.4%	

Salmonella typhi:

The distribution pattern of S. typhi isolates is seen in Table V.

The impression that the Malays are more commonly affected is seen to be statistically significant when they are compared with the Chinese. Older patients appear to have a higher incidence of S. typhi infection with a peak at the 6-10 years age group.

Staphylococcus aureus:

Staph. aureus constituted 4.9% of all isolates. Its distribution is shown in Table VI.

There is no significant difference between incidence in the different racial groups.

The over 10 years old age group does appear to be less affected and this is found to be statistically significant when compared with all the other age groups except the less than a week old.

Shigella:

Shigella constituted 9.3% of all isolates.

The distribution of *Shigella flexner* is given in Table VII.

There is no statistically significant difference between isolation rates in the different racial groups.

The 1-5 years old age group seems to be the most affected and when compared with the less than a year old the difference is found to be statistically significant.

The distribution of *Shigella sonnei* is given in Table VIII.

As in the case of *Shigella flexner* no significant racial differences are seen. Again the 1-5 years old age groups seems to be most commonly affected and this is found to be statistically significant when compared with the less than a year old group and the older than 10 years age group.

2	Tab	le	V	
Distribution	of	S.	typhi	isolates

			Racial groups		
Age group	Malays	Chinese	Indians	Others	Overal
0 - 1/52	0%	0%	0%	0%	0%
< 1 year	0%	0.2%	0%	0%	0.08%
1 – 5 years	0.5%	0%	0%	0%	0.2%
6 - 10 years	2.9%	0%	1.9%	0%	1.6%
> 10 years	1.7%	0%	0.5%	0%	0.8%
overall	1.0%	0.08%	0.3%	0%	

	Tak	ole	VI	
Distribution	of	S.	aureus	isolates

			Racial groups		
Age group	Malays	Chinese	Indians	Others	Overal
1 week	0%	0%	6.3%	0%	0.9%
< 1 year	2.1%	2.0%	0.9%	0%	1.7%
1 – 5 years	2.0%	1.1%	0.5%	0%	1.2%
6 - 10 years	0%	2.1%	1.9%	5.6%	1.6%
> 10 years	0.2%	0.2%	0%	0%	0.1%
overall	1.0%	1.2%	0.6%	0.5%	

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Distribution of S. flexner isolates

. Taka wakazi waka taka			Racial groups		
Age group	Malays	Chinese	Indians	Others	Overal
0 – 1 week	0%	0%	0%	0%	0%
< 1 year	0%	0.2%	0.6%	0%	0.2%
1 – 5 years	1.0%	2.2%	0.5%	0%	1.2%
6 - 10 years	1.5%	0%	0%	0%	0.5%
> 10 years	1.6%	0.2%	1.0%	0.6%	0.9%
overall	1.0%	0.5%	0.7%	0.5%	

			Racial groups		
Age group	Malays	Chinese	Indians	Others	Overal
0 – 1 week	0%	0%	0%	0%	0%
< 1 year	0%	1.0%	0.6%	0%	0.5%
1 - 5 years	3.0%	2.2%	2.1%	0%	2.4%
6 - 10 years	0%	0%	1.9%	0%	0.5%
> 10 years	0.6%	0%	0.7%	0%	0.4%
overall	0.7%	0.8%	1.0%	0%	

Table VIII Distribution of S. sonnei isolates

Enteropathogenic E. coli:

Isolation of enteropathogenic *E. coli* was second only to *Salmonella* despite the fact that they were looked for only in children under 10 years of age. The commonest serotype isolated was 086:K61(B7) (60 isolates), followed by 0119:K69 (B14), 0126: K71(B16), 0128:K67(B12), 026:K60(B6) and 0125: K70(B15) in that order.

The distribution of E. coli serotype 086:B7 is analysed further and shown in Table IX.

The difference in isolation rates between the Chinese and the Indians and Malays is highly significant (p < 0.01) whereas there is no significant difference between the Indians and the Malays.

The highest incidence is noted in the less than a week age group and this is found to be highly significant (p < 0.01) when compared with the other age groups.

The distribution of *E. coli* serotype 0119/B14 is shown in Table X.

The differences seen among the different racial groups is not statistically significant. However again the less than a week age group shows the highest incidence and this is found to be statistically significant when compared with the other age groups.

Mixed Infections:

There were 21 cases of mixed infections accounting for 3.5% of positives. In twenty of these 2 organisms were isolated while there was one case from which *S. typhi*, *S. typhimurium* and *E. coli* 086/B7 were isolated.

Enteropathogenic *E. coli* constituted one of the organisms in 17 of these mixed infections, *Salmonella* serotypes in 16 and *Staph. aureus* in 6. Of the *Salmonella* serotypes, *S. typhimurium* occurred most frequently, being seen in 10 cases.

Table XI shows the detailed breakdown of the cases with mixed infections.

			Racial groups		
Age group	Malays	Chinese	Indians	Others	Overall
1 week	19.2%	7.7%	6.3%	0%	10.0%
< 1 year	1.2%	5.5%	2.0%	0%	3.1%
1 – 5 years	1.5%	6.5%	2.1%	0%	3.4%
6 - 10 years	0%	0%	1.9%	0%	0.5%
overall	1.5%	5.6%	2.1%	0%	

Table IX Distribution of *E. coli* 086:B7 isolates

Age group	Racial groups					
	Malays	Chinese	Indians	Others	Overal	
0 – 1 week	7.7%	6.2%	0%	0%	5.0%	
< 1 year	2.1%	2.5%	1.7%	0%	2.1%	
1 - 5 years	1.5%	1.1%	1.1%	0%	1.2%	
6 - 10 years	1.5%	0%	0%	0%	0.5%	
overall	2.1%	2.3%	1.3%	0%		

Table X Distribution of E. coli 0119/B14 isolates

Table XI Cases of Mixed Infections

Sex	Race		Organisms	
F	Ch	S. bareilly	E. coli 0127/B8	
м	Ch		E. coli 086/B7	Staph. aureus
F	I	S. javiana	E. coli 026/B6	
м	Ch		E. coli 0128/B12	Staph. aureus
F	Ι	S. typhimurium	E coli 0125/B15	
F	Ch		E coli 0114/K90	Sh. sonne
м	Ι	S. habana	E. coli 0119/B14	
м	\mathbf{M}	S. typhimurium	E. coli 0119/B14	
м	Μ	S. anatum	E. coli 0112/K66	
м	Ch		E. coli 086/B7	
F	I		E. coli 0114/K90	
			E. coli 026/B6	
м	$\mathbf{C}\mathbf{h}$	S. typhimurium	E. coli 0119/B14	
м	Ch	S. weltevreden	E. coli 0127/B8	
м	I	S. typhimurium		Staph. aureus
Μ	Ch	S. typhimurium		Staph. aureus
м	Ch	S. typhimurium	E. coli 086/B7	
м	Ch	S. typhimurium	E. coli 086/B7	
		S. typhi		
м	м	S. typhi		
		S. lexington		
F	м	S. typhimurium	E. coli 0128/B12	
м	Ch	S. typhimurium		Staph. aureus
F	Ch	S. typhimurium	E. coli 0119/B14	
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Discussion

A reflection of the role of bacteria as causative agents of diarrhoea may be seen from a study of the isolation rates. In the 3,809 cases studied 15.9%showed the presence of enteropathogenic bacteria. However, it was proven that this isolation rate was age dependent, and was highest in the less than a week age group and decreased with increasing age. It appears therefore that bacteria play a bigger role in the causation of diarrhoea in infants and children while in adults other causes may be more important.

However one point to be considered in explaining this difference in isolation rates between newborns, infants and older age groups is that the younger patients tend to be admitted early to hospital, fresh stools are available and these are taken before antibiotic medication. This increases the chances of bacteria being isolated. In the case of older patients most are treated as outpatients and many have had some measure of treatment outside hospital which may have included antibiotic preparations.

Another point to consider of course is that perhaps in older patients agents other than bacteria themselves are more commonly encountered as causative agents of diarrhoea.

Isolation rates obtained in this study compared favourably with those by other workers (5,6,7,8) who studied diarrhoea in children. Pathogenic bacteria generally account for about 30% of diarrhoea in children (3). Methods employed were very similar and one is led to think that with present techniques only about one third of cases of diarrhoea in children can be attributed to bacteria. This percentage becomes even less when adults are considered and attention must be paid not only to looking for other causes of diarrhoea but also for improving techniques for isolation of a wider range of organisms. Some organisms have only recently been recognised to cause gastroenteritis and special effort must be made to look for them. Examples of this would be Vibrio parahaemolyticus (8), Yersinia enterocolitica (9), Plesiomonas shigelloides (10) and new serotypes of enteropathogenic E. coli which perhaps may need to be looked for in adults as well (11). A group of British soldiers flying from Britain to Aden developed diarrhoea and a new enteropathogenic E. coli serotype was incriminated. The etiological relationship was established when a technician working with the organism a year later developed diarrhoea and the pathogenic strain was isolated from his stool (12). This illustrates the possibility that organisms hitherto unrecognised or looked for as pathogens may in fact be responsible for some of the undiagnosed cases of diarrhoea.

The fact that enteropathogenic E. coli were only looked for in children in this study may also have contributed to the higher isolation rates in the lower age groups.

Chinese in general appear to have a higher isolation rate for bacterial enteropathogens than the other races. This could perhaps be due to the fact that a larger proportion of cases studied belonged to the younger age groups.

Salmonella species formed the largest group of isolates. It was observed after a 3 year study at this Institute in 1955 that the occurrence of Salmonella species in Malaya was quite common and apart from typhoid, members of group B were the ones most frequently found (13). In 1970 it was noted by workers in the Institute for Medical Research that salmonellosis due to serotypes other than S. typhi presented a growing problem second only to typhoid (14). The emergence of serotypes other than S. typhi is further illustrated in the present study where S. typhimurium was by far the predominant organism. Typhoid was only seen about one tenth as frequently as S. typhimurium. Data collected by the W.H.O. Salmonella Surveillance programme for the years 1969-1971 showed that in the European centres frequency of infections associated with other Salmonella serotypes tended to increase whereas those due to S. typhi and S. paratyphi B tended to decrease (15). This therefore appears to be a universal trend. Salmonella serotypes other than S. typhi and S. paratyphi are generally transmitted to man from an animal reservoir through foodstuffs in which a stage of multiplication at normal temperatures is necessary. Their progressive rise in recent years is linked with international traffic in animals and foodstuffs, which is conducive to the introduction of new serotypes, with large scale intensive stock breeding, with industrial food production methods and with communal restaurants which help the spread of Salmonella (16). Hospital cross infection also plays a part in the spread of salmonellosis, particularly in the case of S. typhimurium in paediatric wards. Whatever the organism involved, the infectivity rate is usually high and it is particularly so when infants of the same age group are grouped together.

In the present study racial and age predilections were noted for the commonly occurring Salmonella serotypes. For instance S. typhimurium was seen most commonly in Chinese and in the less than 1 year age group while S. weltevreden affected adults more commonly. S. typhi affected Malays most commonly and its peak of age incidence was the 6-10 year age group. In a review of Salmonella typhi infection in Singapore, it was noted that S. typhi affected mainly the Malays although they formed a minority of the population. The authors attributed this tendency to socio-economic factors (17). The susceptible age group in the Singapore study was found to be between 5-20 year which again is compatible with our findings. Racial and age group differences such as seen in this study could be attributed to many causes including genuine difference in susceptibilities, socio-economic factors, food and cultural habits and the like.

Shigella infections constituted a surprisingly low percentage of isolates (9.3°_{10}) . Improvement in environmental sanitation and standard of living probably explains this decrease and shigellosis in Malaysia today is probably a hand to mouth infection involving carriers connected with the preparation of food (14). A study in Iran showed however that in pre-school children, *Shigella* species were the commonest organisms isolated (6). The same situation was noted in an Indian study (8). These differences are therefore perhaps due to the variation in socio-economic factors.

Another reason that may be put forward for the low incidence of shigellosis in our study is that there is no suitable enrichment medium for *Shigella* and this may have resulted in some cases being missed.

Shigella flexner and Shigella sonnei were the two Shigella species commonly encountered in this study. Similar findings were obtained in a study in India (8) and a previous study at the IMR, Malaysia (14).

While there was no significant differences among the different racial groups with regard to frequency of isolation of *Shigella*, it was noted in the cases of both *flexner* and *sonnei* that the 1-5 year old age group was the most commonly affected while there were no isolates of *Shigella* from patients less than a week old.

Enteropathogenic *E. coli* were looked for in children under 10 years of age. They constituted a major part of the positive isolates in those age groups. Commonest occurring serotypes were 086/B7 and 0119/B14. In the previous study in the I.M.R. (14) the commonest serotypes found were 0128/B12 and 0119/B14. In that study 086/B7 were not looked for initially because the antiserum was not available but was found subsequently in aborigine children. This illustrates the possibility that new serotypes may continuously be found if looked for.

This is further illustrated by the outbreak of infective diarrhoea among babies in hospital at Winchester, England where after screening for the usual pathogens the outbreak was ultimately attributed to an *E. coli* showing resistance to several antibiotics. This strain was subsequently typed as 091 K? H7 (19).

Experimental work on rabbits suggest that disease producing strains may produce a toxic factor responsible for the clinical symptoms while other strains of the same serotype may not (20). This introduces a further problem in looking for incriminating strains of E. coli which are isolated from diarrhoea cases.

Enteropathogenic E. coli have traditionally been looked for in children but recently they have also been incriminated as possible agents of diarrhoea in adults (12).

In the present study, detailed observation of the distribution of *E. coli* 086/B7 and 0119/B14shows that both are significantly common in the less than a week old age group and the incidence appears to decrease as the age increases. 086/B7showed a significant predilection for Chinese patients and perhaps the same factors are involved as in the case of *S. typhimurium*. Diarrhoea due to *E. coli* most often occurs in newly born baby nurseries (3).

There were 21 cases of mixed infection. Mixed infections have also been reported in similar studies conducted by other workers (5,7,8). It is difficult to assess which one or all of the organisms are primarily responsible for the clinical symptoms.

Finding of two pathogens from the stools of diarrhoeal cases may suggest that cross-infection has been highly likely (18).

Summary

3,809 cases of diarrhoea were investigated. In 15.9% of these recognised bacterial enteropathogens were isolated from the faeces. Isolation rates were highest in the less than a week old age group and decreased progressively in the older age group. Isolation rates were highest in Chinese patients. There appeared to be no statistically significant difference in the isolation rates seen in males and females. Salmonella were the commonest encountered.

Significant racial and or age group differences in isolation rates were seen in the case of *S. typhimurium*, *S. weltevreden*, *S. typhi*, enteropathogenic *E. coli* and *Shigella*. Possible explanation for these differences are postulated.

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