

## STAPHYLOCOCCI AND INFANTILE GASTRO-ENTERITIS

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Staphylococci have long been known to be able to cause acute gastro-intestinal disease by means of a relatively heat-stable enterotoxin — an entity clinically recognised as staphylococcal food-poisoning (Wilson and Miles, 1946; Dack, 1959). More recently there have been many reports of diarrhoeal disease secondary to the use of wide spectrum antibiotics (Dearing, 1956; Herplan et al, 1953). Fulminating fatal acute pseudomembranous enterocolitis has also been related circumstantially to the presence of large numbers of Staphylococci in the intestine (Palmer, 1959; Dearing and Needham, 1960). Another entity, "staphylococcal enteritis," in which Staphylococci can be isolated in the stools of patients with diarrhoea using selective media which allow for the growth of Staphylococci, has received attention (Cabrera, et al, 1958; Dearing and Needham, 1960; Lancet, 1961).

The high morbidity rate of infantile gastro-enteritis prompted a survey of the stools from patients under the age of two years admitted with diarrhoea and vomiting, for the presence of coagulase-positive Staphylococci in addition to the other recognised enteric pathogens. The period of investigation extended over 30 months (April 1960 to September 1962), and a total of 1533 specimens were examined.

### Material and Methods

The stools of all children below 2 years admitted with diarrhoea and vomiting to the Isolation Ward of the Paediatric Unit of the General Hospital, Penang, were sent for routine bacteriological diagnosis. The specimens were either stools collected in 2½ oz. screw-capped bottles or rectal swabs in glycerol-saline. These were dispatched to the laboratory a few hours after collection.

In addition to the other media used for identification of enteric pathogens, i.e., *Salmonella*, *Shigella* and *Escherichia* species, the specimen was also put into Robertson's cook-

ed meat media with added 10% sodium chloride and sub-cultured onto ordinary blood agar the following day. Where a **predominant or pure growth of Staphylococci** was obtained, the organisms were tested by the tube method for coagulase activity and the filter paper method for sensitivity to antibiotics (IMR. Report, 1960).

Gram-stained direct smears were examined in random specimens of stools and the findings were related to the results of subsequent culture. Only where **many** Gram positive micrococci were seen, was the smear scored as "Staphylococci present."

### RESULTS

#### Frequency of Isolation of Coagulase-positive Staphylococci.

During the period of this survey, coagulase-positive Staphylococci were isolated in pure or predominant culture on sub-culture from the cooked meat media in 660 instances. This gives an incidence of 43.0%. In 90 cases (6%), the Staphylococci were found together with other known or presumptive intestinal pathogens (Table 1).

#### Age incidence.

Staphylococci were apparently found more frequently in cases of diarrhoea between birth and 3 months than in any other subsequent age group (Table 2).

#### Seasonal Incidence.

There is a tendency to periodicity, with increased frequency of positive isolations during the last trimester of the year (Chan and Lucas, 1964).

#### Correlation of smear to Cultural findings.

Random smears were examined without foreknowledge of the cultural results. These smears were representative of positive and negative cultures. Micrococci were seen in a

TABLE 1

Years	1960	1961	1962	Total
No. of specimens	557	549	427	1533
Staphylococci alone	199	239	132	570
Staph. with Shigella	2	8	13	23
Staph. with Escherichia	13	11	10	34
Staph. with Proteus	19	10	1	30
Staph. with Pseudomonas	1	0	2	3

Coagulase positive Staphylococci Isolations in Gastro-enteritis.

TABLE 2

Age group (months)	0-3	3-6	6-12	12-24	Total
1960	99	40	51	44	234
1961	100	53	68	47	268
1962	64	23	50	21	158

Incidence of Isolated Staphylococci in different Age Groups.

TABLE 3

No. specimens	Culture results	Direct Smear Results			
		Positive	%	Negative	%
129	Positive	67	52.0	62	48.0
203	Negative	39	19.2	164	80.8

Direct Smear Results of Stools for Staphylococci in Gastro-enteritis.

TABLE 4

Antibiotic	No. strains tested	No. Resistant	Per cent.
Penicillin	183	167	91.2
Streptomycin	28	21	75.0
Chloramphenicol	183	108	59.0
Neomycin	28	16	57.1
Bacitracin	28	22	78.6
Tetracycline	154	139	90.2
Oxytetracycline	54	44	81.5
Chlortetracycline	56	8	69.2

Antibiotic Resistant Pattern for Staphylococci Isolated from Stools.

small proportion of smears whose cultures subsequently proved negative for coagulase-positive Staphylococci according to the criteria set.

In contrast the stools that were positive for coagulase-positive Staphylococci on culture had a high proportion of positive smears (Table 3).

### Sensitivity to Antibiotics

Even though almost all the patients had their specimens sent immediately on admission to hospital and very few had been hospitalised previously, a remarkable finding was the large number of antibiotic resistant strains (Table 4). Over 90% of the isolated strains were resistant to penicillin and tetracycline.

### DISCUSSION

Since Koch first demonstrated the presence of micrococci in pus in 1878, the *Staphylococcus* continues to be a bacteriological problem even in our modern antibiotic era. Its relation to suppurating lesions is well-known, but the fact that it can be associated with diarrhoea following the use of wide-spectrum antibiotics and also its circumstantial association with cases of fatal pseudo-membranous enterocolitis, has alerted workers to the possibility of intestinal *Staphylococcal* disease existing as a distinct entity. The reason why this problem has not been as fully investigated, is probably due to the failure to recognise the possibility of Gram positive organisms causing diarrhoea, and consequently no great efforts were made to isolate these organisms. A parallel could be drawn from the history of enteropathogenic *Escherichia coli* which was considered as early as 1927 by Azam to be of etiological significance in infantile gastroenteritis, but had to wait twenty years before they were looked for as causes of outbreaks of diarrhoea in infants (Taylor, 1960). Another important factor lies perhaps in the methodology adopted by most laboratories doing culture of stools for pathogens. Selective media for Gram negative organisms are usually used and *Staphylococci* can hardly be expected to compete for bacteriological recognition (Cabrera et al. 1958) under such circumstances.

The higher overall incidence of *Staphylococci* in the intestinal contents of infants is well-known. Martyn (1949 b) found virtually the same incidence of *Staphylococci* in stools of infants with diarrhoea (44.5%) and controls (46%) and "concluded, therefore, that there is no evidence that *Staphylococci* bear any causal relationship to infantile diarrhoea and vomiting" (Elek 1959). But later, other workers have pointed out that the presence of a *Staphylococcus* in faeces — as distinct from

predominant or pure growth — is not a criterion for infection. Dearing et al (1953) reported that the presence of resistant strains of *Staphylococci* in more or less pure culture in the intestinal tract may produce mild or extremely severe gastrointestinal and systemic symptoms and that treatment with a drug to which the organism is sensitive leads to clinical cure (Dearing 1956).

It has been shown (Dearing and Needham, 1950; Cabrera et al, 1958); by the use of appropriate methods, that there is a significant relation between the isolation of *Staphylococci* and diarrhoea — an entity which is recognised as "*Staphylococcal enteritis*." Hinton et al. (1960), who did a survey of 826 adults and 346 infants with and without diarrhoea, came to the conclusion that there was a high carrier rate in the stools, especially in those who were exposed to hospital environments. Nevertheless, they concluded that the simple demonstration of a *Staphylococcus* in the intestinal content of any specific person seems to be "an observation of very limited significance" and a positive culture of a coagulase positive *Staphylococcus* only means that the patient is in a statistical group in which the disease may occur." On the other hand, the observation that these organisms are found as predominant members of the faecal flora "should be treated with considerable respect." It is perhaps significant to note in their reports, that, a "3+" load of *Staphylococci* was found in the stools of 5.7% of infants without symptoms compared to 14.1 — 25.1% in those with diarrhoea. The corresponding figures for a "4+" load were 0.0% and 3.0 — 7.8%. In other words *Staphylococci* were three to five times as commonly isolated in these two groups where there is symptomatic infection. Our positives correspond roughly to their classification of a "3+" or "4+" load.

The difficulty attendant on incrimination of the *Staphylococcus* as an enteropathogen is the difficulty in the demonstration of a specific biological effect on the intestine of animals. Recognition of an enterotoxin in *Staphylococcus* contaminated milk and its effect in humans was described by Barber as early as 1914 in the Philippines (quoted by Wilson and Miles, 1946). More recently, Surgalla and Dack (1955) and Prohaska (1959) have demon-

strated the ability of Staphylococci isolated from antibiotic-treated patients and from cases of pseudomembranous enterocolitis to cause intestinal disease in the rhesus monkey and chinchillas, thus demonstrating the enteropathogenicity of the Staphylococcus experimentally. Attempts at typing of isolated Staphylococci with phages have not produced conclusive evidence regarding a specific strain responsible for diarrhoea (Dearing and Needham, 1960; Cabera et al 1958). Sensitivity tests have not helped in this respect either. The antibiotic resistance pattern has not, in our experience, been found to be significantly different from other Staphylococci isolated in routine work. Thus from the diagnostic point of view, there seems to be no readily available in vitro tests to differentiate the enteropathogenic from other coagulase-positive Staphylococci.

From the public health point, it is perhaps necessary to realise the implication of finding antibiotic resistant Staphylococci in the stools of infants with gastro-enteritis. The dangers of faecal contamination of carriers and bed-clothes are no less than those due to Salmonella or Shigella (Greendyke et al., 1958). This is an important consideration in the nursing of such cases. Further, as Dearing et al. (1953) suggest "antibiotic agents should be used only when they are specifically indicated and should not be used promiscuously for treatment of minor illnesses."

It has also been pointed out (Lancet, 1961) that if direct smears show large numbers of Gram positive cocci in clumps, actual intestinal infection with Staphylococcus aureus is highly probable. Our studies have shown that in cases where coagulase-positive Staphylococci were subsequently isolated in large numbers with the use of selective media, there was a significant number of positive smears.

Perhaps an objection to our findings could be that we have used a selective method for the isolation of Staphylococci. But, selective media are used in the identification of other pathogenic enterobacteria. We realise that the present evidence does not signify an invariable causal relationship between the Staphylococcus and infantile gastroenteritis; but the findings are suggestive and point to the necessity for

further work on this subject. In the meantime, it is perhaps rational to exhibit appropriate therapeutic measures in the treatment of infantile gastro-enteritis.

### Summary

Coagulase-positive **Staphylococci** were isolated in predominant or pure culture, by the use of selective methods, in 43.0% of 1533 cases of infant gastroenteritis. The causal relationship is probable, and specific measures directed against the organism are indicated in the management of these cases.

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