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Plesiomonas shigelloides in South Australia

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SYNOPSIS *Plesiomonas shigelloides*, a motile, oxidase-positive, Gram-negative rod that may possess shigella antigens, was isolated from the faeces of 36 children and two adults. In 13 children the organism was thought to be possibly the cause of enteritis and in eight children there was no evidence of intestinal disease. In nine children with gastroenteritis other enteropathogenic bacteria were found in addition, and six children had intestinal disease due, or possibly due, to other factors. Only four of the 38 strains had shigella antigens.

From the faeces of a baby, severely ill with gastroenteritis, we isolated in 1963 a shigella-like bacterium. The severity of the illness, the presence of serum O agglutinins against the organism to a titre of 1 in 32, and our inability to find another cause of the disease prompted us to send the isolate to the Shigella Reference Laboratory, Colindale, where Dr K. Patricia Carpenter identified our isolate as *Plesiomonas shigelloides* and drew our attention to this species of whose occurrence and significance we had previously been unaware.

Ferguson and Henderson (1947) were the first to describe the organism and they called their strain C 27. The most popular name for the organism seems to be *Aeromonas shigelloides* (Cowan and Steel, 1965) but, as we believe that there are a number of features distinguishing it from other *Aeromonas* species, we prefer and use the name *Plesiomonas shigelloides* (Habs and Schubert, 1962).

MATERIALS AND METHODS

From March 1963 through to September 1967, 38 patients had *Ples. shigelloides* cultured from their faeces. Sampling of colourless colonies from Oxoid Leifson's desoxycholate citrate agar and Oxoid S.S. agar provided the isolates of *Ples. shigelloides*. Leifson's agar was inoculated by direct plating of faeces and also from G N broth (Edwards and Ewing, 1962) after overnight incubation, while S.S. agar was inoculated from Rappaport broth (Rappaport, Konforti, and Navon, 1956) after overnight incubation.

The amount of growth on primary plating was described as scanty when there were few colonies (up to 10) present, a moderate growth indicated 10 to 20 colonies, and a heavy growth meant that the organism dominated the flora.

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Hydrolysis of casein, fibrinolysin, and lipase activity were tested for by the methods of Eddy (1962). Other bacteriological characteristics were determined by routine methods (Cruickshank, 1965). Oxoid Multodisks were used for antibiotic sensitivity testing. In a few patients serum O and H agglutinins against the patient's endogenous strain of *Ples. shigelloides* were studied by standard techniques (Cruickshank, 1965).

RESULTS

PATTERN OF ISOLATIONS Isolates have been found with increasing frequency in our laboratory. In 1963 and 1964 only one case occurred, while there were seven cases of each in 1965 and 1966 and 23 in the first nine months of 1967. No cases were seen in the colder months of July to October and most cases occurred in the hotter months of January to April.

AGE OF PATIENTS There were 36 children in the series and, although their ages ranged from 4 weeks to 11 years, the tendency was for younger children to be involved. Thus one-third were less than 3 months, two-thirds were less than 2 years, and most (91·7%) were under 6 years. A nurse of 18 and a medical registrar of 26 constituted the two adults in the series.

CLINICAL FEATURES The nurse had an illness lasting one day with diarrhoea and some vomiting and the one faeces cultured yielded, on enrichment, *Ples. shigelloides*. The medical registrar had moderately severe diarrhoea and some vomiting for three days and the symptoms abated in five days. Scanty *Ples. shigelloides* was cultured on direct plating of the first faecal specimen and two subsequent specimens obtained during convalescence were negative.

Serum O agglutinins were present to a titre of 1 in 64 and four weeks later the titre was unchanged.

The 36 children can be arranged into four clinical groups. The largest group of 13 children had *Ples. shigelloides* isolated at the beginning of an episode of enteritis and there was no other discernible cause of their symptoms (cases 1 to 13, Table I). Diarrhoea was the dominant symptom and although in the majority it was the only symptom, five children had vomiting. All children were admitted to hospital, but in nine children the disease was not severe, while in three children dehydration was sufficient to require intravenous fluid therapy. All recovered. In eight children in this group there was growth on primary plating and in six this was scanty in amount but cases 2 and 8 showed heavy growth. In case 7 we had no record of the method of isolation and in the remaining four cases (cases 3, 4, 12, and 13) the isolate was obtained by enrichment only.

The second group consisted of eight children in whom there was no evidence of intestinal disease and the isolate had been detected in one routine faecal culture done on admission to hospital (Table II). In two patients (cases 14 and 17) enrichment provided the isolate and in the remainder scanty growth occurred on primary plating with the exception of case 18 in which a moderate growth was obtained.

In the third group there were nine children with gastroenteritis from whom bacteria, conventionally regarded as enteropathogenic, were isolated in addition to *Ples. shigelloides* (Table III). A scanty growth of *Ples. shigelloides* on primary plating occurred in four patients (cases 22, 23, 27, and 30); in case 29 there was no record of the method of isolation and in the rest enrichment only was positive. From five children *Ples. shigelloides* was isolated at the beginning of their illness and yet in four of these the disease was mild. From four children *Ples. shigelloides* was isolated during the course of their illness and in none was there evidence that the organism caused an exacerbation of symptoms. *Shigella sonnei* was the commonest accompanying enteric pathogen.

Finally a fourth miscellaneous group of six children had gastrointestinal symptoms (cases 31 to 36, Table I). In three children there were other factors that may have contributed to their symptoms and these other factors were oesophageal reflux in case 31, *Giardia lamblia* in case 33, and radiotherapy for abdominal lymphosarcoma in case 35. In a further two children

TABLE II

PLESIOMONAS IN CHILDREN WITH NO EVIDENCE OF INTESTINAL DISEASE

Case No.	*Age*	*Diagnosis*
14	1 mth	Irritability, no definite diagnosis
15	2 mth	Anaemia, post-Rh incompatibility
16	1 yr	Upper respiratory tract infection
17	2 yr	Parental neglect, bronchopneumonia, ear infection
18	4 yr	Infectious mononucleosis
19	5 yr	Ocular herpes
20	8 yr	Self-limited undiagnosed arthritis
21	10 yr	'Malaise', no definite diagnosis

TABLE I

PLESIOMONAS SHIGELLOIDES IN CHILDREN WITH ENTERITIS

Case No.	*Age*	*No. of Faecal Cultures*	*No. Positive for* Ples. shigelloides	*Comments*
1	6 wk	1	1	Mild disease, given neomycin
2	7 wk	3	3	Mild disease, given neomycin
3	2 mth	2	1	Spina bifida and hydrocephalus, mild enteritis for 1 week, no antibiotics
4	2 mth	31	1	Mild diarrhoea: acquired in hospital, osteomyelitis and salmonella enteritis
5	2 mth	11	3	Severe diarrhoea for 1 week, required I.V. therapy and antibiotics
6	2 mth	1	1	Favism in remission, mild diarrhoea, no antibiotics
7	2 mth	6	5	Mild disease, no antibiotics
8	3 mth	12	1	Severe diarrhoea and vomiting for 2 weeks, required I.V. therapy and antibiotics. Serum O agglutinins 1 in 32 and 1 in 16 one week later
9	3 mth	3	1	Mild disease, no antibiotics
10	9 mth	4	3	Mild disease, no antibiotics
11	9 mth	16	5	Acute severe diarrhoea, required I.V. therapy and antibiotics. No O or H agglutinins. Had iron-deficiency anaemia
12	1 yr	3	2	Mild disease, no antibiotics
13	1 yr	4	3	Moderately severe diarrhoea for 5 days with passage of blood, settled with neomycin
31	3 mth	2	1	Some vomiting and loose bowel actions, oesophageal reflux present
32	1 yr	11	3	Mild diarrhoea. Plesiomonas isolated on final hospital days when 8 previous faecal cultures were negative
33	2 yr	1	1	Mild diarrhoea, mild infection with *Giardia lamblia*
34	4 yr	5	3	Abdominal pain and vomiting for five days. Serum agglutinins to Plesiomonas absent on admission and one week later
35	4 yr	1	1	Mild diarrhoea treated with chloramphenicol. Radiotherapy, given for abdominal lymphosarcoma, possible cause of enteritis
36	11 yr	11	5	During an exacerbation of ulcerative colitis 5 out of 11 faeces yielded *Ples. shigelloides*. Serum O agglutinins to Plesiomonas 1 in 128, no H agglutinins. Slow response to neomycin

TABLE III

PLESIOMONAS IN CHILDREN INFECTED WITH ACCEPTED INTESTINAL PATHOGENS

Case No.	*Age*	*Other Infection on Admission*	*Other Hospital-acquired Infection*	*No. of Faecal Cultures*	*No. Positive for* Ples. shigelloides	*Hospital Day when Plesiomonas Isolated*	*Comments*
22	6 wk	*E. coli* O26:B6	—	10	1	1st	Severe diarrhoea
23	6 mth	*Salm. derby*	—	17	3	1st to 3rd	Mild disease
24	2 yr	*Salm. tymphimurium*	—	4	1	1st	Mild disease
25	1 yr	*Sh. sonnei*	—	8	2	1st, 2nd	Hydrocephalus, mild diarrhoea
26	3 yr	*Sh. sonnei*	—	6	2	1st	Mild disease
27	3 yr	*Sh. sonnei*	*Salm. typhimurium on* 10th day	33	4	18th to 21st	No symptoms due to *Ples. shigelloides*
28	1 yr	*Sh. sonnei*	*E. coli* O127:B8 on 22nd day	12	2	41st to 42nd	No symptoms due to *Ples. shigelloides*
29	4 mth	Pseudomonas empyema	Necrotizing pseudomonas enteritis 2nd to 4th hospital weeks	24	1	70th	No symptoms or serum agglutinins due to *Ples. shigelloides*
30	2 mth	*E. coli* O127:B8	—	8	1	17th (final day)	No symptoms due to *Ples. shigelloides*

(cases 32 and 34) we doubted the significance of the *Ples. shigelloides* but we suspect that the organism caused an exacerbation of diarrhoea in the boy of 11 with ulcerative colitis (case 36). This last patient was the one case in this group from whom a heavy growth of *Ples. shigelloides* was found on direct plating. In two others (cases 31 and 34) enrichment only was positive, no record was available for case 32, and in cases 33 and 35 there was scanty growth on primary plating.

There were no deaths in the series.

METHODS OF ISOLATION In 33 instances in the 36 children we had noted whether the isolate was obtained by direct plating on Leifson's agar or by prior enrichment in G N and/or Rappaport broth. Of these 33 instances direct plating was positive in 21, and in the 12 instances in which only the broth cultures were positive, both broths were positive four times, Rappaport alone positive once, and G N broth alone positive seven times. In 13 instances we could compare a positive direct plating with parallel enrichment in the broths. In these 13 instances, both broths were positive twice, Rappaport alone positive twice, and G N broth alone positive eight times. The superior enrichment medium was G N broth.

BACTERIOLOGICAL FEATURES *Ples. shigelloides* was a Gram-negative rod that formed, on Leifson's agar after overnight incubation, colourless translucent convex colonies 1 mm in diameter. In none of these features was it distinctive. In Kligler iron agar a shigella-like reaction occurred with an acid butt, alkaline slope, and without swarming, odour, gas, or sulphide production. There was good growth on blood agar after overnight anaerobic incubation. Table V gives other bacteriological features.

Only four of the 38 strains possessed shigella antigens. The strains from cases 233, 1, and 36 were agglutinated by Commonwealth Serum Laboratory *Shigella sonnei* phase I antiserum to its full titre of 1 in 640. Another strain (case 22) was agglutinated by *Shigella flexneri* type 6 antiserum to its full titre of 1 in 320.

TABLE IV

BACTERIOLOGICAL FEATURES OF PLESIOMONAS SHIGELLOIDES

Test	*No. Positive out of No. Tested*
Motility	38/38
Acid in glucose (aerobic, 1 day)	38/38
Acid in glucose (anaerobic, 1 day)	38/38
Gas in glucose	0/38
Acid in lactose (1 day)	1/38
Acid in lactose (2-7 days)	37/38
Acid in sucrose (1 day)	0/38
Acid in maltose (1 day)	38/38
Acid in mannitol (1 day)	0/38
Acid in dulcitol (1 day)	0/38
Acid in inositol (1 day)	37/37
Acid in adonitol (1 day)	0/5
Acid in salicin (1-2 days)	0/38
Acid in salicin (3-28 days)	38/38
H_2S (Kligler)	0/38
Cytochrome oxidase	38/38
Indole	38/38
Simmon's citrate	0/38
Urease	0/38
Phenylalanine deaminase	0/37
Lysine decarboxylase	38/38
Arginine dihydrolase	35/38 (of 35 positive, 3 weak)
Ornithine decarboxylase	38/38
Methyl red (37°C)	38/38
Voges-Proskauer (37°C)	0/38
Gluconate	0/37
Haemolysis	0/38
Gelatin (1 week)	0/38
Casein hydrolysis	0/38
Fibrinolysin	0/38
Lipase	0/38

ANTIBIOTIC SENSITIVITY Table VI shows that strains tended to be 'antibiotic sensitive' rather than 'antibiotic resistant'.

TABLE V

ANTIBIOTIC SENSITIVITY OF PLESIOMONAS SHIGELLOIDES

Antibiotic	*No. Sensitive out of No. Tested*
Ampicillin	21/38
Hetacillin	6/11
Streptomycin	31/35
Neomycin	37/38
Chloramphenicol	35/38
Tetracycline	32/34
Furazolodine	27/27
Sulphonamide	25/38

DISCUSSION

Ples. shigelloides and *Aeromonas* species are motile, aerobic, Gram-negative rods that are oxidase positive, show good anaerobic growth, and have a fermentative attack on glucose. These properties separate them from other bacteria of medical importance. The features that distinguish *Ples. shigelloides* from *Aeromonas* species are the ability of Plesiomonas to produce acid in inositol and its inability to produce haemolysis, lecithinase, lipase, fibrinolysin, rapid gelatin liquefaction, hydrolysis of casein, serum digestion, and acid in mannitol (Eddy, 1960; Eddy and Carpenter, 1964).

A striking feature in the descriptions of *Ples. shigelloides* has been its possession of *Shigella sonnei* phase I antigen (Ferguson and Henderson, 1947; Schmid, Velaudapillai, and Niles, 1954; Sakazaki, Namioka, Nakaya, and Fukumi, 1959; Eddy and Carpenter, 1964) and of the 54 strains described by these workers, 31 possessed *Shigella sonnei* phase I antigen. The organism may possess other shigella antigens, for in Japan, Hori, Hayashi, Maeshima, Kigawa, Miyasato, Yoneda, and Hagihara (1966) described 10 strains that had *Shigella dysenteriae* type 7 antigen. Although we infrequently found shigella antigens, our findings confirm that the sonnei antigen is not the one exclusively found. It will be interesting to see, as more evidence comes to hand, whether the capacity of the organism to cause disease can be correlated with the possession of different shigella antigens. In addition, these serological features mean that the rapid presumptive identification of *Shigella* species by reaction in Kligler iron agar and agglutination by specific antisera must be preceded by the exclusion of *Ples. shigelloides*. Motility and oxidase reaction are rapid tests that are useful in this regard.

Ples. shigelloides has been isolated from the faeces of healthy man, from adults and children with a history of or contact with diarrhoea, from patients excreting *Shigella sonnei*, and from many animals, including the dog, cat, monkey, sheep, goat, and cow (Schmid *et al*, 1954; Sakazaki *et al*, 1959; Eddy and Carpenter, 1964; Geizer, Kopecký, and Aldová, 1966). The organism has been implicated in outbreaks of food poisoning and is coming to be commonly regarded as a potential cause of gastroenteritis (Sakazaki *et al*, 1959; Hori *et al*, 1966).

Heavy growth of *Ples. shigelloides* on primary plating occurred in three of the 33 children for whom we had kept records of the method of isolation. Two of the three children came from the first group with possible Plesiomonas enteritis (cases 2 and 8) and the third child had ulcerative colitis but was thought to have an exacerbation of diarrhoea due to *Ples. shigelloides* (case 36). In the remaining 30 children primary plating showed an equal tendency to be positive in the presence of enteritis (eight out of 14 positive) and in the absence of enteritis (six out of eight positive). In children infected with accepted intestinal pathogens primary plating was less frequently positive (four out of eight positive).

Our own experience is consistent with the view that, although the organism may be found in the absence of enteritis and although it may occur in the presence of intestinal disease, especially dysentery, *Ples. shigelloides* may be the cause of enteritis, particularly in infants. The enteritis it appears to cause is usually but not always mild.

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