# *PLESIOMONAS SHIGELLOIDES* ASSOCIATED WITH DIARRHOEAL DISEASE IN MALAYSIAN CHILDREN

Y S Lim
L J Young
S Balakrishnan

**Bacteriology Laboratory**
**Department of Pathology**
**Sultanah Aminah General Hospital**
**Johore Baru**
**Malaysia**

Y S Lim, MSc
Bacteriologist

L J Young
Medical Laboratory Technologist

**Department of Paediatrics**
**Sultanah Aminah General Hospital**
**Johore Baru**
**Malaysia**

S Balakrishnan, MBBS, FRCP
Consultant Paediatrician

## SYNOPSIS

*Plesiomonas shigelloides* **was isolated from 5 (2.1%) of the 234 children with diarrhoea and none of the 230 controls. In one child, the organism was found in association with** *Salmonella*. **Two strains had** *Shigella sonnei* **phase I antigen. All the strains were susceptible to the aminoglycosides, cephalosporins, nalidixic acid, nitrofurantoin, chloramphenicol and cotrimoxazole; but resistant to the penicillins. Alkaline peptone water enrichment subcultured to desoxycholate citrate agar proved to be a useful method for isolating this organism from faeces. As the role of** *P. shigelloides* **in causing gastrointestinal disease remains controversial, further studies are necessary to determine its enteropathogenicity.**

## INTRODUCTION

*Plesiomonas shigelloides* was first isolated from the faeces of a patient by Ferguson and Henderson (1) in 1947. They called this bacterium C27, a motile organism which possessed *Shigella sonnei* phase I antigen. It was later given the name *Aeromonas shigelloides* and eventually classified as a new genus *Plesiomonas*, and named *Plesiomonas shigelloides* (2).

The isolation of *P. shigelloides* from patients with diarrhoea has been reported by many investigators (3,4,5,6,7,8,9,10,11). Clinical descriptions of infection due to cases of *P. shigelloides* suggest that it may cause diarrhoea in otherwise healthy persons. However, several attempts at identifying an enterotoxin produced by this organism have been unsuccessful (11,12,13).

There have been few reent reports to compare the prevalence of *P. shigelloides* in patients with diarrhoea with the prevalence in controls without gastrointestinal symptoms. The present study was undertaken to study *Plesiomonas*-associated gastroenteritis in hospitalized children in Johore Baru, Malaysia, where no previous study was done. Isolates were also tested for their *in vitro* susceptibilities to various antimicrobial agents.

## MATERIALS & METHODS

### Patients

A total of 234 infants and children up to the age of 10 years who were admitted to the Sultanah Aminah General Hospital in Johore Baru with acute diarrhoea were studied from December 1985 to November 1986. Included in this study as controls were 230 children admitted with illnesses other than gastroenteritis. Of the 234 children with diarrhoea, 123 were boys and 111 were girls whereas the corresponding numbers in the control group were 129 and 101, respectively.

### Stool Examination

Stool specimens were inoculated onto desoxycholate citrate agar (DCA; Oxoid), MacConkey agar (MAC; Oxoid) and alkaline peptone water (APW) (pH 8.6) enrichment broth. After overnight incubation at 37° C, subcultures were made from APW to DCA and MAC. All culture plates were incubated at 37° C and examined after overnight incubation (14). All oxidase-positive colonies and those typical of *P. shigelloides* were confirmed biochemically (15). Serotyping of the strains with commercially prepared *Shigella* antisera (Wellcome Diagnostics) was then performed (6).

Faecal samples from children with diarrhoea were also examined by standard bacteriological techniques (16) to isolate and identify *Salmonella*, *Shigella* and enteropathogenic *Escherichia coli* (EPEC).

The comparative disc-diffusion method of Stokes and Waterworth (17) was used to test the antibiotic sensitivities of the strains isolated, using Mueller Hinton agar (Oxoid). Antimicrobial agents tested included ampicillin (10 mcg), carbenicillin (100 mcg), amikacin (30 mcg), tobramycin (10 mcg), cephaloridine (30 mcg), cefuroxime (30 mcg), cefoperazone (75 mcg), cefotaxime (75 mcg), ceftazidime (30 mcg), nalidixic acid (30 mcg), nitrofurantoin (100 mcg), tetracycline (30 mcg), polymyxin B (300 units), chloramphenicol (30 mcg) and cotrimoxazole (25 mcg). All the antimicrobial discs, except for amikacin (BBL), were obtained from Oxoid.

## RESULTS

Table 1 shows the isolation rates of bacterial enteropathogens from the stools of children with and without diarrhoea. From the children with gastroenteritis, 5 samples (2.1%) were positive for *P. shigelloides*, 36 samples (15.4%) were positive for EPEC, 25 samples (10.7%) were positive for *Salmonella* and 13 samples (5.6%) were positive for *Shigella*. None of the 230 stool samples from the control group yielded *P. shigelloides*. *Salmonella* was isolated as the other intestinal pathogen from one of the *P. shigelloides*-positive sample.

Two of the 5 strains possessed *Shigella sonnei* phase I antigen. All the isolates grew on DCA after APW enrichment. Two strains also grew on DCA whereas 2 other strains were also isolated from MAC, before and after the enrichment, respectively.

Susceptibility of the isolates to antimicrobial agents showed that all strains were susceptible to the aminoglycosides, cephalosporins, nalidixic acid, nitrofurantoin, chloramphenicol and cotrimoxazole; but resistant to the penicillins. Two strains were resistant to polymyxin B whereas 3 strains exhibited susceptibility to tetracycline.

## DISCUSSION

In the present study, *P. shigelloides* was isolated from 5 (2.1%) diarrhoeal children. Workers in India (8,9), Japan (10) and Thailand (11) have isolated this organism in 0.5 to 37.5% of diarrhoeal cases. From our control group, none of the stool specimens showed the presence of *P. shigelloides*. The recorded prevalence of *P. shigelloides* carriers has been very low. In Czechoslovakia (18), only 0.1% of the 10,643 control subjects were carriers whereas in Thailand (11), 25 out of 451 (5.5%) were found to harbour this organism. In a survey of 38,454 healthy subjects in Japan (19), only 3 were found to be carriers.

Of the 5 children with gastroenteritis from whom *P. shigelloides* was recovered, *Salmonella* was also isolated from the stool of a child. Mixed infections with another enteric pathogen do occur and have been observed by other investigators (6,9). Cooper and Brown (6) reported that EPEC, *Salmonella* and *Shigella* were isolated from 9 children in addition to *P. shigelloides*, with *Shigella sonnei* being the commonest accompanying enteric pathogen.

The observation that *P. shigelloides* possessed *Shigella sonnei* phase I antigen was first reported by Ferguson and Henderson (1). Since then, other investigators have reported similar findings (3,6,20). Two of our isolates were found to possess *Shigella sonnei* phase I antigen. The organism may possess other *Shigella* antigens. In Japan, Hori et al (5) described 10 strains that had *Shigella dysenteriae* type 7 antigen whereas in Australia, Cooper and Brown (6) isolated a strain which was agglutinated by *Shigella flexneri* type

**TABLE 1**
**ISOLATION OF BACTERIAL ENTEROPATHOGENS FROM CHILDREN WITH AND WITHOUT DIARRHOEA**

| Group | No. tested | No. of isolates (% of samples submitted) | | | |
|---|---|---|---|---|---|
| | | Enteropathogenic | | | |
| | | *P. shigelloides* | *E. coli* | *Salmonella* | *Shigella* |
| Children with diarrhoea | 234 | 5 (2.1) | 36 (15.4) | 25 (10.7) | 13 (5.6) |
| Children without diarrhoea | 230 | 0 (0) | ND* | ND | ND |

*ND = Not done

6 antiserum. As *P. shigelloides* and *Shigella* give similar reaction in Triple Sugar Iron agar (TSI), the rapid presumptive identification of *Shigella* by the TSI reaction and agglutination by specific antisera should be preceded by the exclusion of *P. shigelloides* biochemically. Two simple and rapid tests, namely, the oxidase reaction and motility, are recommended for this purpose (6).

All our strains were sensitive to the aminoglycosides, cephalosporins, nalidixic acid, nitrofurantoin, chloramphenicol and cotrimoxazole, but resistant to the penicillins. This is in broad agreement with studies done by other workers (6,15).

Like von Graevenitz and Bucher (14), we have found APW a useful enrichment medium for *P. shigelloides*. However, work done by Millership and Chattopadhyay (21) showed no evidence that isolation was aided by enrichment in APW. If APW enrichment had been omitted in our study, 3 of the isolates would have been missed as only on 2 occasions, direct plating onto DCA also yielded *P. shigelloides*. MAC was found to be less sensitive than DCA in recovering *P. shigelloides* as it was only isolated twice on MAC.

As attempts to identify an enterotoxin produced by *P. shigelloides* have been unsuccessful (11,12,13), its role in causing gastrointestinal disease in otherwise healthy persons remains unclear. There is a need for a well-controlled study of volunteers to investigate whether *P. shigelloides* strains isolated from diarrhoeal stool can produce symptoms in healthy subjects. Research work to determine the sources of infection and the risk factors for illness should be conducted. The development of more effective assays for characterizing enterotoxins may prove useful in establishing this organism as an etiological agent of gastroenteritis.

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