

may most often result in disease, such as in small children and visitors to endemic areas. Identifying the virulence properties of *Aeromonas* that are associated with diarrheal illness will lead to further refinement in clinical and epidemiological studies.

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Overview of four U.S. Navy overseas research studies on *Aeromonas*

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The United States Navy conducts research on medically important infectious diseases at five laboratories within the United States Naval Medical Research and Development Command. One laboratory is Naval Medical Research Institute in Bethesda, Maryland, with a detachment in Peru. Naval Medical Research Unit No. 2 is in Manila, the Philippines, with a detachment in Jakarta, Indonesia. Naval Medical Research Unit No. 3 is in Cairo, United Arab Republic of Egypt. Data presented here were gathered during population surveys and investigations of acute diarrhea, and are focused on the role of *Aeromonas* spp. and *Plesiomonas shigelloides* in enteric disease.

1) Studies in Jakarta from July 1981 through March 1982 evaluated 1695 patients with diarrhea; 338 patients with enteric fever but not diarrhea served as controls. *Aeromonas hydrophila* was

present in 11% of patients with diarrhea and 4% of the controls ($p < 0.001$). *A. sobria* was present in 3% with diarrhea and 0.6% of controls ($p < 0.05$). *P. shigelloides* was present in 0.4% with diarrhea and not present in controls (not statistically significant due to small number of isolates). *A. hydrophila* was the only potential pathogen isolated in 75 of the 182 individuals and was associated with *Vibrio cholerae* in 90 and other organisms in 17. *A. sobria* was the only pathogen in 25 of the 51 and was isolated with *V. cholerae* in 12 and other organisms in 14. *P. shigelloides* was isolated alone in 5 of the 7 individuals. Cultures were done directly from the fecal sample and again after overnight enrichment in alkaline peptone water (APW). Agars used were MacConkey, Hektoen Enteric, Thiosulfate-citrate-bile salts sucrose and *Salmonella-Shigella*.

Table 1. Prevalence of etiological agents of diarrhea at San Lazaro Hospital, Philippines, in 1983/1984

Organism	Percent positive 1983		1984	
	Patients (n = 1021)	Controls (n = 201)	Patients (n = 1884)	Controls (n = 375)
<i>Aeromonas hydrophila</i>	2**	0.5	1**	0.3
<i>Plesiomonas shigelloides</i>	1	0	0	0.3
<i>Salmonella</i> spp.	12	6	8	5
<i>Shigella</i> spp.	10	0.5	13	2
Enterotoxigenic <i>Escherichia coli</i>	8	5	2*	0.3
<i>Vibrio cholerae</i>	4	0	4	0.5
Non-ol <i>V. cholerae</i>	4	0	2	0.3
Other <i>Vibrio</i> spp.	2	0	0	0.3
<i>Campylobacter</i> spp.	4	2	1	0.3
Rotavirus	27	6	33	5

* Not tested in patients less than 20 years of age after March 1984; ** $p = 0.05$ vs controls (p calculated only for *Aeromonas* and *Plesiomonas*).

Table 2. Antimicrobial resistance of *Aeromonas* spp. and *Plesiomonas* spp. isolated in the Philippines and Peru

Organism	Percent resistant Ampicillin	Tetracycline	Chloramphenicol	SXT*	Erythromycin
<i>Aeromonas</i> spp. Philippines (n = 37)	57	22	16	24	8
Peru (n = 199)	98	16	28	16	85
<i>Plesiomonas shigelloides</i> Philippines (n = 33)	24	42	0	0	3
Peru (n = 21)	76	10	19	10	100

* SXT - Sulfamethoxazole-Trimethoprim.

Table 3. Age-specific isolation rates for *Aeromonas* in Mogadishu, Somalia

Age	Percent isolation Acute diarrhea	Asymptomatic control
0-6 months	7 (n = 54)	0 (n = 27)
7-12 months	11 (n = 92)	4 (n = 24)
13-24 months	19 (n = 62)*	3 (n = 27)
25-36 months	8 (n = 38)	9 (n = 24)
3-14 years	15 (n = 44)	5 (n = 41)
0-14 years	12 (n = 290)*	4 (n = 143)

* Isolation significantly associated with acute diarrhea ($p < 0.01$).

Table 4. Bacteria isolated from individuals with acute diarrhea in Peru

Organism	Percent positive Peruvian adults (n = 335)	Peruvian children (n = 410)	American adults (n = 150)
<i>Campylobacter jejuni</i>	2	12	9
<i>Salmonella</i> spp.	11	4	1
<i>Shigella</i> spp.	9	14	15
<i>Plesiomonas shigelloides</i>	3	1	2
<i>Aeromonas</i> spp.	12*	12**	5***
<i>Vibrio</i> spp.	2	1	1
Enterotoxigenic <i>Escherichia coli</i>	1	10	13

* 33%, ** 39%, and *** 55% Presented with another potential pathogen.

2) A second study of secretory diarrhea in Indonesia evaluated 196 individuals with diarrhea believed to be due to cholera. Of the 34 individuals shown to have *Aeromonas* spp., 7 had *Aeromonas* spp. as the only pathogen; it was associated with *V. cholerae* or other vibrios in the other 27 patients. Culture and enrichment were done in the same way as in the previous study.

3) In Manila during 1983 and 1984, patients with diarrhea were evaluated, as were controls. Results are shown in table 1. *Aeromonas* spp. comprised only 2% of the identified potential etiological agents in 1983, compared to 0.5% in controls. In 1984 *Aeromonas* spp. were identified in 1% of patients compared to 0.3% of controls. *P. shigelloides* comprised 1% of identified etiological agents in 1983 and 1984 while controls had only 1 isolation in the 2 years. Cultures were done directly from fecal samples and after overnight enrichment in APW and Selenite F Broth. Agars used were the same as in the previous studies but included *Yersinia* selective media. Table 2 gives disc diffusion susceptibilities (Kirby-Bauer method) for *Aeromonas* spp. and *P. shigelloides* isolated in the Philippines and in Peru.

4) A study of diarrhea in children up to 14 years of age in Mogadishu, Somalia showed that *Aeromonas* spp. isolations were significantly associated with acute diarrhea ($p < 0.05$) as compared to controls. Age-specific isolation rates of *Aeromonas* spp. varied from 7% to 19%, as shown in table 3. *Aeromonas* (14%) spp. were more frequently isolated from patients arriving at a primary health care clinic compared to *Shigella* spp. (5%) while the reverse was true of patients seen at a hospital (5% *Aeromonas* spp. to 18% *Shigella* spp.). Cultures and enrichments were done as previously described in the Manila study.

5) Population surveys of apparently healthy individuals in Peru revealed positive fecal cultures for *Aeromonas* spp. in 4% of coastal inhabitants (n = 880) and 6% of jungle inhabitants (n = 268). *P. shigelloides* was only found in 1% of the jungle inhabitants. Fecal cultures were done on 132 young people from England, Australia, Canada and the United States when they arrived in Peru before spending two months working on Operation Raleigh projects in the Peruvian jungle. *Aeromonas* spp. were present in 5% and *P. shigelloides* in 1%. Upon leaving the jungle, 73 individuals were recultured; 10% had *Aeromonas* spp. ($p = 0.05$), 4% had *P. shigelloides* ($p > 0.05$), 7% had *Shigella* spp. and 14% had *Campylobacter jejuni*.

Studies of individuals with acute diarrhea in Peru are summarized in table 4. *Aeromonas* spp. were present in 12% of Peruvian adults or children with diarrhea, but were associated with other

recognized pathogens 30-40% of the time. *Aeromonas* spp. were present in 5% of American adults with diarrhea, but were associated with another pathogen 55% of the time. *P. shigelloides* was present in 1-3% of the individuals with diarrhea and was rarely associated with other pathogens.

6) A study of fever and/or diarrhea occurring in U.S. Navy and Marine Corps personnel during a goodwill tour of South America and West Africa in 1985 identified 166 clinically symptomatic individuals. Diarrhea was present in 144. *Aeromonas* spp. were present in 5 individuals and *P. shigelloides* in 6, accounting for 17% of the total agents identified. Cultures in all the Peru-based studies were done on ampicillin-trypticase soy agar as well as on the previously described media. Enrichment was done in APW. Antibiotic resistance by disc diffusion for *Aeromonas* spp. and *P. shigelloides* isolated in Peru and from the shipboard study are shown in table 2.

It is clear that the association of *Aeromonas* spp. and *P. shigelloides* with acute diarrhea has been documented by these U.S. Navy studies in various areas of the world. There is a need for research to conclusively determine the causality between these organisms and the clinical presentation. The frequent combination of *Aeromonas* spp. with other enteric pathogens needs to be explored. The apparent predilection of *Aeromonas* spp. gastroenteritis in young children in developing countries and in individuals transiting these areas (travelers' diarrhea) makes this organism important to the military's research efforts in enteric diseases.

If this workshop can produce a generally acceptable determination of specific research required to clearly define the role of *Aeromonas* and *Plesiomonas* as etiologies of diarrheal diseases, a great advance will have been made to create order out of confusion.

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