

Three-Year Prospective Study of Intestinal Pathogens in Madrid, Spain

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During the period July 1980 through June 1983, in a General Hospital in Madrid, the following organisms were detected from 6,970 patients with gastroenteritis: 710 *Salmonella* spp., 506 *Campylobacter jejuni*, 379 *Shigella* spp., 12 *Yersinia enterocolitica*, 1,466 rotavirus, 134 *Giardia lamblia*, and 4 *Entamoeba histolytica*. Chloramphenicol showed good activity against most tested strains of *Salmonella* spp., *Shigella* spp., and *C. jejuni*. The incidence of *Salmonella* spp. and *Shigella* spp. was very marked in the hot dry months of the year, rotavirus predominated during the cold months, and no seasonal variations of importance were seen for *C. jejuni* and *G. lamblia*.

The risk of acquiring a diarrhea continues to be a major problem for people who travel to certain areas of the world (13). Spain is a country with more than 40 million visitors each year, many of whom prefer to come during the hot months of the year.

From July 1980 to June 1983 we studied 6,970 diarrheal stools from different patients in a Madrid General Hospital which covers a potential population of 1,200,000. Of the samples, 40% were from outpatients, and the rest were from inpatients. The male to female ratio was 1:1.

The feces were inoculated onto conventional media for the isolation of *Salmonella* spp., *Shigella* spp. (McConkey agar and salmonella-shigella agar), *Campylobacter jejuni* (Skirrow blood agar) (12), and *Yersinia enterocolitica* (Schieman CIN agar; Oxoid Ltd.) (10). The remainder of the sample was frozen at -30°C for subsequent investigation of rotavirus by an enzyme-linked immunosorbent assay procedure (Enzygnost; Behring Institute). Specific media for the isolation of *Vibrio cholerae* or *Clostridium difficile* were introduced only when necessary. A total of 120 feces from children less than 3 years old were tested for heat-labile toxin-producing *Escherichia coli* by the Biken test (6), but owing to the low proportion of positive findings (two strains), this test was subsequently abandoned. Wet mounts of 5,022 freshly passed stools were examined for leukocytes or parasites or both. The samples which were not immediately processed were stored at 4°C for a maximum of 36 h.

The susceptibility of *Salmonella* spp., *Shigella* spp., and *C. jejuni* to ampicillin, chloramphenicol, fosfomicin, erythromycin, and trimethoprim-sulfamethoxazole (TMP-SMZ) was determined by the agar dilution method of Ericsson and Sherris (5). The medium used for TMP-SMZ was Iso-Sensitest (Oxoid Ltd.). MIC was defined as the lowest concentration of antibiotic that inhibited development of visible growth.

The following organisms were diagnosed: 710 *Salmonella* spp. (10.2%), 506 *C. jejuni* (7.3%), 379 *Shigella* spp. (5.4%), 12 *Y. enterocolitica* (0.2%), 1,466 rotavirus (21%), 134 *Giardia lamblia* (2.7% of 5,022 samples), and 4 *Entamoeba histolytica*. The susceptibilities of *Salmonella* spp., *Shigella* spp., and *C. jejuni* to the five antimicrobial agents are shown in Table 1. Chloramphenicol was the most active agent

against the species tested. The most frequently isolated species of *Salmonella* were *S. typhimurium* (38%), *S. enteritidis* (27%), *S. heidelberg* (6%), *S. blockley* (5%), *S. agona* (3%), *S. infantis* (3%), *S. ohio* (3%), and *S. typhi* (3%). For *Shigella* species the most frequent isolate was *S. sonnei* (92%), followed by *S. flexneri* (7%) and *S. boydii* (1%). The seasonal distribution of the different etiologies is shown in Fig. 1. Figure 2 represents the distribution of the sample and the different organisms according to age. In viral and parasitic diarrhea, the male to female ratio was 1:1, but in bacterial diarrhea it was 3:2.

Rotavirus is the most common cause of infectious diarrhea in Madrid; in the current study it reached an overall frequency of 21% and a year peak (mean of the 3 years) of 28%. This rate is less than that in other countries (4, 7), possibly owing to the climatic conditions of Spain and the fact that the population in our study included adults. On the other hand, our isolation rates of *Salmonella* spp. and *Shigella* spp. yielded results similar to those in other Mediterranean

TABLE 1. Susceptibility of intestinal pathogens

Bacteria (no. of isolates) and antibiotic tested	MIC ₉₀ ^a	Range
<i>Salmonella</i> spp. (162)		
Ampicillin	4	0.5-64
Chloramphenicol	4	2- \geq 128
Fosfomicin	32	4-64
Erythromycin	128	8- \geq 128
TMP-SMZ	6.2	3.1-25
<i>C. jejuni</i> (132)		
Ampicillin	64	2- \geq 128
Chloramphenicol	8	1-16
Fosfomicin	64	8-128
Erythromycin	2	0.1-64
TMP-SMZ	50	1.5- \geq 100
<i>Shigella</i> spp. (136)		
Ampicillin	64	2-64
Chloramphenicol	4	2- \geq 128
Fosfomicin	64	8- \geq 128
Erythromycin	128	8- \geq 128
TMP-SMZ	100	1.5- \geq 100

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^a Concentration required to inhibit 90% of the strains tested.

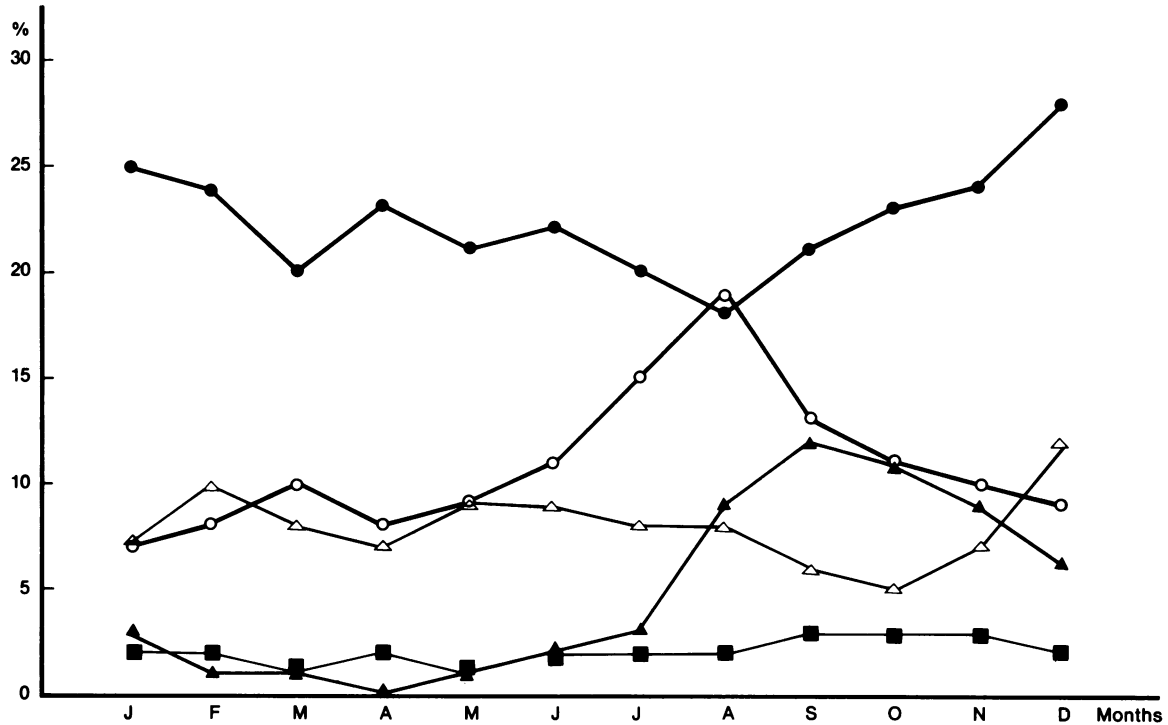


FIG. 1. Monthly distribution of organisms. Mean of 3 years. Percentages are rounded to the nearest integer. Symbols: ●, rotavirus; ○, *Salmonella* spp.; △, *C. jejuni*; ▲, *Shigella* spp.; ■, *G. lamblia*.

countries (G. F. Abbate, I. Alagia, V. Leonesa, and P. Altucci, Abstr. Int. Congr. Chemother. 13th, Vienna, SE 7.3/1, part 75, p. 1-4, 1983), but were higher than those in North America (3).

Our *Campylobacter* isolates are included in the range (4 to 14%) observed in other developed countries (3, 9). *G. lamblia*, heat-labile toxin-producing *E. coli*, and especially *Y. enterocolitica* were infrequently detected from unselected sporadic diarrhea samples. Three of the four cases of *E. histolytica* infection were in patients who had returned from overseas. Nearly 1% of the patients showed two simultaneous potential pathogens, mostly rotavirus and *Salmonella* spp. or rotavirus and *C. jejuni*.

The antibiotic susceptibility of *Salmonella* spp. and *C. jejuni* was similar to that found in other reports (11). *Shigella sonnei* is almost always resistant to ampicillin and TMP-SMZ (1, 8), which traditionally have been used by pediatricians in the treatment of diarrhea. Of every 10 *Shigella* spp., 9 were *S. sonnei*, slightly higher than in other reports (2). The incidence of *Salmonella* spp. and *Shigella* spp. was very high in the hot dry months of the year; the incidence of *Shigella* spp. peaked 1 month later than that of *Salmonella* spp. Rotavirus predominated during the cold, or perhaps wet, months of the year, although the unremitting drought of the last 4 years in Madrid, Spain, makes it difficult to draw clear distinctions. *C. jejuni* and *G. lamblia* had more uniform occurrences throughout the year.

Half of the patients with diarrhea were less than 12 months old. In relation to this sample, patients in the first year of life were those most likely to acquire rotavirus; in the second year the most frequent pathogen was *C. jejuni*, whereas in the third year the most frequent pathogens were *G. lamblia* and *Salmonella* spp. *S. typhi* was never isolated from children less than 1 year old. Children 4 to 7 years old were the most likely to contract shigellosis.

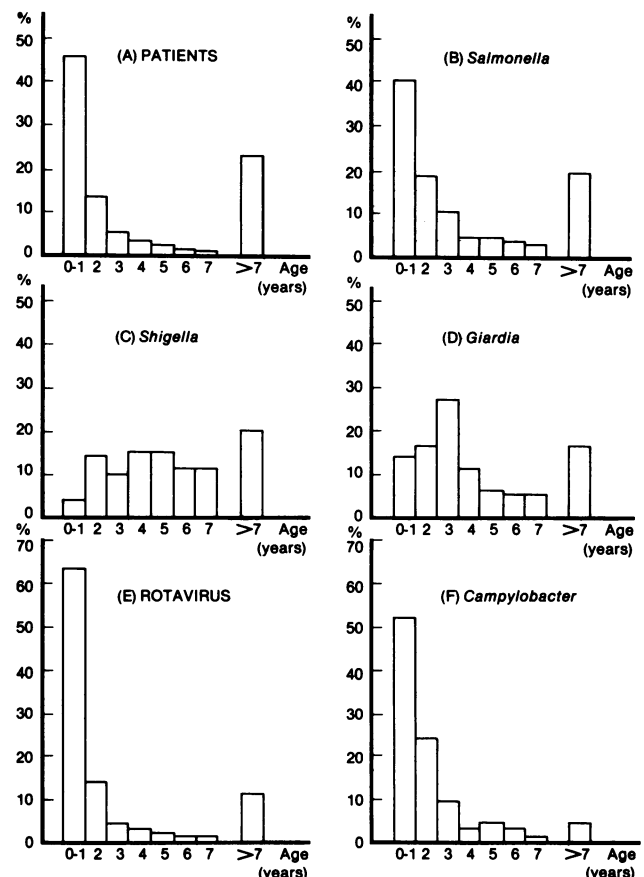


FIG. 2. Age distribution of total patient sample (A) and by etiologic agents (B through F).

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