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Original Article

A study on the antibiotic resistance of *Shigella*

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ABSTRACT

Background The hospital morbidity caused by *Shigella* or dysentery ranges between 0.3 to 2.9%. Irrational use of antibiotics causes a persistent diarrhea and may lead to drug resistance.

Objectives With various kinds of antibiotics available in Indonesia at the moment, this study aimed to anticipate the kinds of antibiotics appropriate for shigellosis and to evaluate the clinical spectrum of dysentery in children in Indonesia.

Method The study involved 50 children diagnosed with dysentery or dysentery-like syndrome, aged 1 to 12 years, who came to four different hospitals in Jakarta, from November 2001 to April 2002. Parents were asked for their consent. Interviewers recorded details of the children's history of illness and the physical examinations. Stool culture and resistance tests were done.

Results Fifty dysentery cases, comprising 30 males and 20 females, 98% aged from 1 to 5 years, came to the four hospitals during the study period. Only 24 cases had positive *Shigella* cultures, of which 87% were *Shigella flexneri* and 17% were *Shigella sonnei*. The clinical manifestations of shigellosis were bloody stools (83%), mucus in the stool (75%), and watery diarrhea (96%). Fever and tenesmus were absent in 67% and 92% of subjects, respectively. Almost 87% of shigellosis cases were resistant to cotrimoxazole; all were sensitive to colistin and most were sensitive to nalidixic acid.

Conclusion This data suggests that colistin and nalidixic acid are drugs of choice for dysentery syndrome. The clinical manifestation of dysentery is not always accompanied by bloody stools but mostly incorporates watery diarrhea and mucus in the stool **[Paediatr Indones 2005;45:49-54].**

Keywords: *dysentery, shigellosis, children, antibiotics, antibiotic resistance, antibiotic sensitivity*

Diarrheal diseases remain a major health problem in Indonesia, as apparent in its high morbidity, mortality, and the numerous outbreaks which often cause deaths and unrest within the community. Based on data taken from several surveys in Indonesia, approximately 280 persons out of every 1000 in the population suffer from diarrhea each year.[1] A household survey conducted in the provinces of West Nusa Tenggara, South Sumatera, and Yogyakarta in 1992 revealed that roughly 10-20% of deaths in under-fives were caused by bloody diarrhea.[2] Data on diarrheal outbreaks in 1997 noted that 14 provinces had already been infected by this disease, which covered 38 districts and a total of 15,192 victims and 138 deaths (CFR 0.91%).[3] Hospital morbidity caused by *Shigella* or dysentery ranges between 0,3 to 2,9%.[1]

Irrational use of antibiotics causes a persistent diarrhea and may lead to drug resistance. Several reports show that some shigellosis cases are resistant to

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cotrimoxazole.[4-6] With various kinds of antibiotics available in Indonesia at the moment, it is necessary to anticipate the kinds of antibiotics appropriate for *Shigella* or dysentery and to evaluate the clinical spectrum of dysentery or shigellosis in Indonesia.

Methods

The study involved 50 children diagnosed with dysentery or dysentery-like syndrome. Dysentery syndrome is characterized by occurrence of diarrhea, fever, abdominal pain, tenesmus, and passage of stools that usually contain mucus, pus, and blood. Study subjects consisted of children aged 1 to 12 years who came to four different hospitals in Jakarta (Cipto Mangunkusumo Hospital, Sulianti Saroso Hospital for Infectious Disease, Fatmawati Hospital, and UKI Hospital) from November 2001 to April 2002. The subjects' parents had been informed of the nature of the study and asked for their consent. Interviewers recorded details of the children's history of illness. Clinical examination of the children, including measurement of weight and height, were performed.

Fresh stool specimens from all children were collected either by rectal swab or by swabbing of freshly produced stool, both performed using a sterile cotton swab. The stool-containing swabs were placed in Stuart agar transport media, and the inoculated media were refrigerated at 4^{o}C after being labeled. Specimens in transport medium were transferred to the laboratory in insulated boxes with refrigerant packs designed to ensure refrigeration at 4^{o}C. Transfer of specimens to the microbiology laboratory took place daily. Each transfer was accompanied by a transfer log form. Isolation of *Shigella* was done using Gram negative (GN) broth, and then plated to MacConkey agar and *Salmonella* chromagar with streaking for isolated colonies. The *Shigella* cases were subjected to serogrouping and were tested for resistance to several antibiotics available in Indonesia, such as ampicillin, cotrimoxazole, nalidixic acid, the newer quinolones, and ceftriaxone.

Statistical analysis was done using SPSS 10.0 for Windows. Data were presented as mean (SD). The chi-square test was used to compare nominal data. A P value of <0.05 was considered statistically significant.

Results

During the period of study, each hospital involved was expected to collect 30 stool specimens, but at the end of the study period none of the four hospitals could reach this target. Cipto Mangunksumo Hospital collected only 14 specimens, Sulianti Saroso Infectious Disease Hospital 22 specimens, Fatmawati Hospital 12 specimens, and UKI Hospital only 2 specimens. Thus, a total 50 specimens were collected from four hospitals.

Among 50 dysentery patients, there were 30 males and 20 females, 49 aged between 1 to 5 years and 1 aged 5 to 10 years. Mean age was 20.9 (SD 7.03) months. There was no statistically significant difference between males and females in the chance for acquiring shigellosis. *Shigella* infection was significantly more prevalent among under-fives ($P<0.05$). Twenty-three out of 50 cases have received antibiotic treatment prior to the study, and this condition significantly affected the culture results of patients included in this study ($P<0.05$) (**Table 1**).

There were only 24/50 cases with stool cultures positive for *Shigella*. In the others, 4/50 cases yielded pathogenic *E. coli* and 1/50 yielded *Salmonella*. In the remaining cases (11/50), cultures were negative. The species of *Shigella* involved were *Shigella flexneri* (21/50) and *Shigella sonnei* (3/50). No *Shigella dysenteriae* or *S. boydii* was found (**Figure 1**).

The clinical manifestations of shigellosis found in this study were mucus in the stool in 18/24 patients and watery stools in 23/24. Blood in the stool, tenesmus, and fever were absent in 20/24, 22/24, and 16/24 patients, respectively. Among patients with negative cultures for Shigella, 22/26 had bloody diarrhea, 26/26 had mucus in the stool, 18/26 had watery stools, 14/26 had tenesmus, and 19/26 had fever. A statistically significant difference between *Shigella*-positive and *Shigella*-negative patients was found for all symptoms except fever.

From 24 cases in which stool cultures were positive for *Shigella*, 82% were resistant to ampicillin, 84% to sulphametoxazole, 82% to chloramphenicol, and 96% to tetracycline. In contrast, 100% of cases were sensitive to colistin, 96% to nalidixic acid, 100% to ceftriaxone, 100% norfloxacin, 96% to aztreonam, and 100% to ciprofloxacin (**Figure 2**).

TABLE 1. CHARACTERISTICS OF STUDY SUBJECTS

Characteristic	n	%	
Number of patients	50	100	
Age			Mean : 22.9 (SD 7.03) months
1 – 5 years	49	98	
>5 – 10 years	1	2	
>10 years	0	0	
Sex			x^2(McNemar): 0.053;df=1; P=0.817
Male	30	60	
Female	20	40	
Prior antibiotic treatment			x^2(McNemar): 8.194;df=1; P=0.004
Yes + positive culture	6	12	
No + positive culture	18	36	
Yes + negative culture	17	34	
No + negative culture	9	18	

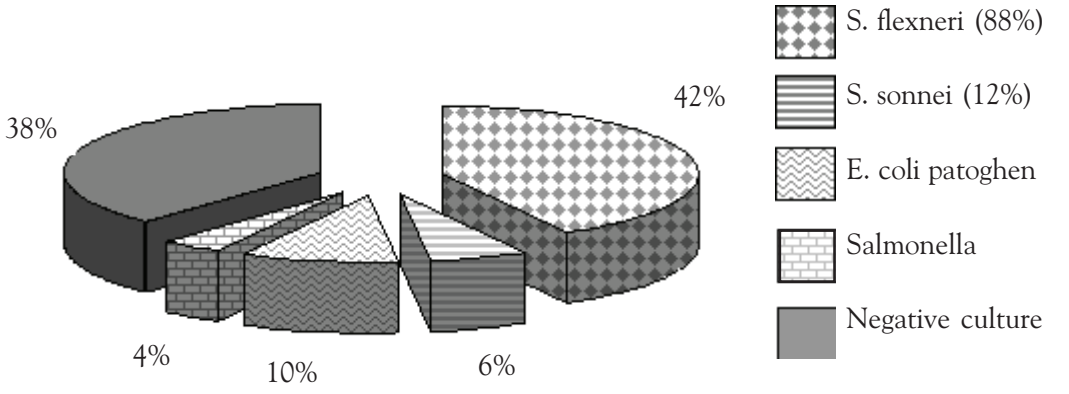

FIGURE 1. DISTRIBUTION OF STOOL CULTURE PATHOGENS AND STRAINS OF SHIGELLA

Discussion

There were several limitations in the hospital-based collection of shigellosis cases in this study. Besides the low hospital morbidity (0.3-2.9%),[2] the prior use of antibiotics in nearly half the cases influenced stool culture results ($P<0.05$), possibly precluding a positive culture even though the symptoms of shigellosis were typical.

The symptomatology induced by *Shigella* is variable and ranges from very mild to extremely violent, involving seizures and hyperthermia. The incubation period is 1 to 4 days, but may be as long as 8 days with *S. dysenteriae*. Young children are more susceptible, although no age group is immune to infection.[7] The majority of *Shigella*-infected individuals have a benign, self-limited clinical course lasting 5 to 7 days. In some children, the clinical course will be more severe, with fever, abdominal pain, malaise, watery or bloody diarrhea, and tenesmus.[3]

Several lines of evidence indicate that wild-type *Shigella* infection confers protective immunity. In endemic areas, the incidence of shigellosis peaks during the first 5 years of life and declines thereafter, suggesting that immunity develops after repeated exposures during childhood.[8] This is consistent with the result of this study, which shows that shigellosis was most frequent in the age group of 1 to 5 years, without any significant difference between male and female patients ($P=0.817$). Most cases in this study were mild ones, with watery diarrhea but without tenesmus.

Shigellosis is a biphasic illness, with an initial phase characterized by watery diarrhea with small in-

TABLE 2. CLINICAL SPECTRUM OF THE SUBJECTS

Clinical spectrum	Shigella culture Positive (n / %)	Negative (n / %)	n	
Blood				x^2(McNemar):23.09;df=1; P=0.00
Yes	4 / 17	22 / 85	26	
No	20 / 83	4 / 15	24	
Mucus				x^2(McNemar):7.39;df=1; P=0.07
Yes	18 / 75	26 / 100	44	
No	6 / 25	0 / 0	6	
Watery				x^2(McNemar):5.98;df=1; P=0.14
Yes	23 / 96	18 / 69	41	
No	1 / 4	8 / 31	9	
Tenesmus				x^2(McNemar):11.88;df=1; P=0.01
Yes	2 / 8	14 /54	16	
No	22 / 92	12 / 46	34	
Fever				x^2(McNemar):7.94;df=1; P=0.05
Yes	8 / 33	19 / 73	27	
No	16 / 67	7 / 27	23	

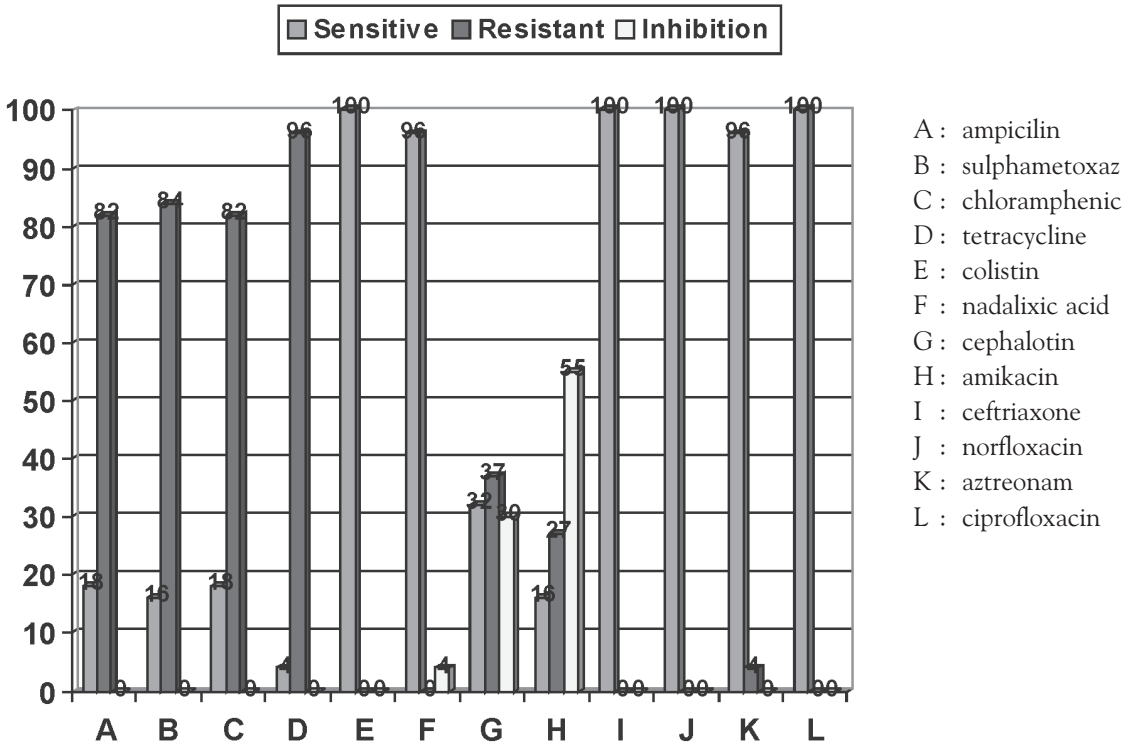

FIGURE 2. RESULTS OF ANTIBIOTIC RESISTANCE TESTS TO *SHIGELLA*

testine secretion. This may be evident in a carefully taken history of patients with shigellosis. In a classic study, monkeys given *Shigella* orally experienced an early secretory diarrhea, whereas monkeys inoculated with the organisms per rectum had only colitis and no watery diarrhea. In volunteer studies, this phase of considerably watery diarrhea was found to last for about 18 hours. The watery nature of stools is caused by *Shigella* enterotoxins, which elicit classic secretory responses. There are two new toxins that are secreted

by *Shigella*. *Shigella* enterotoxin 1 is found only in *S. flexneri* 2A; it is encoded by a chromosomal gene and it seems to have the same A1B5 subunit structure as cholera toxin or shiga toxin. The other toxin, *Shigella* enterotoxin 2, encoded by a gene found on the virulence phase of *Shigella*, and it is present in all serotypes. In the later phase of illness, the diarrhea is scanty, characterized by scanty bloody mucoid stools.[9]

Besides *Shigella*, other causes of dysentery syndrome were found in this study, such as pathogenic *Escherichia coli* and *Salmonella*. Shiga toxin-producing *E. coli* (STEC) is a group of *E. coli* strains (most notably serotype O157:H7) capable of causing significant human disease. Over 90% of STEC infections present as enteric disease ranging from mild secretory diarrhea to severe hemorrhagic colitis.[10] Unfortunately we did not do further examination to detect the strain of *E. coli* found in this study.

During 1990-2000, resistance to ampicillin increased from 70% to 90%, co-trimoxazole from 77% to 85%, chloramphenicol from 71% to 77%, streptomycin from 71% to 79%, and nalidixic acid from 0% to 11.3%. Resistance to tetracycline decreased from 89% but with MIC50 and MIC90 values still outside the susceptible range.[11] Consistently, in our study we found high resistance rates to ampicillin, sulphamethoxazole, chloramphenicol, and tetracycline. These results suggest that these antibiotics should not be used as first line drugs in the treatment of shigellosis. Nalidixic acid should still be selectively used for treatment, while ciprofloxacin and ofloxacin can be ideal alternatives.[11] This study also found that all cases were still sensitive to colistin *in-vitro*.

Given the ease of transmission and propensity to cause life-threatening illness, antibiotic treatment is always indicated for *Shigella* infections. Antibiotic therapy for shigellosis has evolved since the 1960s as *Shigella* infections have become resistant to the drugs of choice from preceding eras. In the 1990s quinolones emerged as the preferred agents for treatment of *Shigella* infection. All available quinolones have excellent *in-vitro* activity, and multiple trials support their clinical efficacy. Nevertheless, quinolone therapy has its limitations. None of the newer fluoroquinolones is approved for use in children, although there is some published experience using a quinolone for serious *S. dysenteriae* type 1 infection in pediatric patients.[12] It would be reasonable to use a quinolone regimen for a child with severe shigellosis, particularly if empirical non-quinolone therapy has failed and antibiotic sensitivity testing does not provide an obvious alternative.[13] Given the concerns about their safety in children, the search for other agents continues. First- and second-generation cephalosporins are active *in-vitro* but disappointing in clinical use. Studies using cefixime, an oral third-generation cephalosporin, in Turkish[14] and American[15] children using daily cefixime for 5 days appeared promising.

Finally, we conclude that colistin and nalidixic acid are drugs of choice for dysentery syndrome, and that the clinical manifestation of dysentery is not always accompanied by blood but mostly present watery, mucus-containing stools. The rapid emergence of multiple antimicrobial resistance and the difficulty to control the spread of the disease solely by improvement of sanitation underlines the importance of efforts to develop safe and effective vaccines.

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