SEROVAR DISTRIBUTION AND ANTIBIOTIC SUSCEPTIBILITY OF NONTYPHOIDAL *SALMONELLA* ISOLATED FROM PEDIATRIC PATIENTS IN JAKARTA, INDONESIA

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Abstract. A study was conducted during January 2003 through August 2005, at two community health centers in south Jakarta, Indonesia, to detect nontyphoidal *Salmonella* infections in children with diarrhea. A total of 814 rectal swab samples were collected, of which 56 (6.9%) were positive for *Salmonella*. Among the serovars detected, *Salmonella enterica* serovar Typhimurium was found most frequently in 32.1% of all *Salmonella* isolates. Antimicrobial susceptibility testing using eight antibiotics showed 5.6% to 66.7% of *Salmonella* serovars resistant to ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, and nalidixic acid. However, all serovars were susceptible to norfloxacin, ciprofloxacin, and ceftriaxone.

INTRODUCTION

Diarrheal disease is an important global problem that causes high rates of morbidity and mortality in developing countries, and although mortality from infectious diarrheal diseases has progressively been reduced, there has been a substantial increase in morbidity among young children around the world (Guerrant *et al*, 2002; Kosek *et al*, 2003). Diarrheal diseases occur most frequently in the conditions of poor environmental sanitation and hygiene, inadequate water supplies, poverty, and limited education, as found especially in developing countries (Black, 2001). Among the bacteria causing diarrhea, *Salmonella* continues to be a major public health problem. Nontyphoidal salmonellosis and typhoid are clearly the most economically important foodborne diseases, and while the incidence of typhoid is stable, cases of nontyphoidal salmonellosis are increasing worldwide (Olsen *et al*, 2001). Infection with nontyphoidal *Salmonella* usually results in a self-limiting gastroenteritis that does not require antibiotic therapy. However, serious sequellae, including systemic infection and death, sometimes may occur (Mead *et al*, 1999; Su *et al*, 2004).

Over the last two decades, the incidence of nontyphoidal *Salmonella* infections has increased considerably and reached epidemic level in several countries (Fierer and Swancutt, 2000). More than 95% of *Salmonella* infection cases are food-borne, and salmonellosis accounts for 30% of deaths resulting from foodborne illness in the United States (Mead *et al*, 1999). Recently, specific serovars were linked with certain foods or exposures. For example, outbreaks of *Salmonella* serovar Enteritidis have been repeatedly associated with raw or undercooked eggs (Mohle-Boetani *et al*, 1998) and *S.* Marina infection has been associated with exposure to reptiles (Mermin *et al*, 1997). In the late 1990s, *S.* Typhimurium and *S.* Enteritidis were most frequently isolated, account-

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ing for over 50% of isolates from patients in the United States (Hohmann, 2001). In addition, various *Salmonella* serovars resistant to conventional antibiotics such as ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and other newer antibiotics (quinolones and extended-spectrum cephalosporins) have been reported with increasing frequency in many areas of the world (Su *et al*, 2004).

However, there is very little information available from Indonesia regarding the status of *Salmonella* in association with diarrheal diseases. The prevalence of *Salmonella* serovars and their distribution among diarrheal patients, especially those in children, is not known because although most laboratories performed isolation and identification procedures for *Salmonella,* serotyping is infrequently performed. Identification of *Salmonella* serovars and its pattern of antimicrobial susceptibility may provide epidemiological data for proper antimicrobial therapy. In an attempt to obtain this information we conducted a *Salmonella* study, and the results are reported herein.

MATERIALS AND METHODS

Subjects and sample collection

During the period of January 2003 through August 2005, a stool survey was performed on pediatric patients (aged 0-14 years) presenting with diarrhea who were seen at two community health centers in south Jakarta, Indonesia.

Criteria used in the screening of diarrheal patients for study inclusion purposes were: children presenting with three or more loose stools in the previous 24 to 72 hours. Voluntary, written, and informed consent was obtained from the parent or guardian of the patient prior to the enrollment in this study. Clinical information was obtained by the investigators or nurse, including the duration, frequency and consistency of diarrhea and other related symptoms. After the interview, the nurse collected a rectal swab sample before antibiotic treatment was administered. The swab was placed in Cary-Blair transport medium and refrigerated immediately. The rectal swab samples were transported in an icebox to the Microbiology Laboratory of the Medical Faculty of Trisakti University in Jakarta the following day.

Laboratory processing

Upon arrival at the laboratory, rectal swab samples were processed immediately. The swab was inoculated directly onto MacConkey (MAC) agar, *Salmonella-Shigella* (SS) agar and Xylose Lysine Desoxycholate (XLD) agar. The swab was then placed into mannitol selenite broth (MSB) for *Salmonella* culture enrichment. All culture media were purchased from DIFCO (Becton Dickinson, MD). Agar plates and enrichment broth were incubated aerobically at 37ºC for 20-24 hours.

Cultures in MSB were inoculated onto SS and XLD plates and the plates were incubated aerobically at 37ºC for 20-24 hours. Non-lactose fermenting colonies of *Salmonella* on MAC, SS, and XLD were selected and tested for their biochemical reactions on Kligler's iron agar, sucrose semisolid agar, and motility-indole-ornithine medium. Specific antisera were used for serologic characterization of the isolates (Bopp *et al*, 2003). Antimicrobial susceptibility testing was performed by the standard disk diffusion method (NCCLS, 1997; 2001) using ampicillin, chloramphenicol, tetracycline, trimethoprim-sulfamethoxazole, ceftriaxone, nalidixic acid, norfloxacin, and ciprofloxacin (Beckton Dickinson, MD).

RESULTS

Fifty-six (6.9%) of 814 rectal swab samples obtained from pediatric cases with diarrhea during the project yielded *Salmonella.* The isolates consisted in decreasing order of *Salmonella* group B (2.3%), group C2 and D (1.4%, each), group E (1.1%), and group C1

Table 1
Distribution of *Salmonella* serovar in pediatric patients.

Isolate Group/serovar	Age group			
	Number of isolates (%)			
	0 - 11 mo	1 - 4 yr	5 - 14 yr	Total
	n=452	n=311	n=51	n=814
B				
Typhimurium	11 (2.4)	5 (1.6)	2 (3.9)	18 (2.2)
Derby	1 (0.5)	0	0	1 (0.1)
C1				
Virchow	4 (0.9)	2 (0.6)	0	6 (0.7)
C2				
Hadar	4 (0.9)	4 (1.3)	0	8 (1.0)
Newport	2 (0.4)	1 (0.3)	0	3 (0.4)
D				
Enteritidis	5 (1.1)	4 (1.3)	2 (3.9)	11 (1.4)
E				
Weltevreden	5 (1.1)	0	1 (2.0)	6 (0.7)
Lexington	3 (0.7)	0	0	3 (0.4)
Total	35 (7.7)	16 (5.1)	5 (9.8)	56 (6.9)

(0.7%). The serovar distribution is shown in Table 1. No *Salmonella enterica* serovar Typhi nor *S*. Paratyphi were isolated. *S.* Typhimurium was isolated from cases of all age groups, and along with *S.* Enteritidis was the most prominent in patients 5-14 years old (3.9%). *S.* Hadar with a 1.0% isolation rate was the third most isolated serovar. However, *Salmonella* group C1 and C2 were not found in children age 5-14 years old, they were only found in 0-4 year old patients. Among the age group of 0-11 months old, *Salmonella* group B was found in 2.9% of patients followed by *Salmonell*a group E (1.8%) and *Salmonella* group C2 (1.3%) with *S.* Typhimurium (2.4%), *S*. Weltevreden (1.1%), and *S*. Hadar (0.9%).

Diarrheal children who were brought to the two community health centers in south Jakarta were age 0-11 months (452 patients), 1-4 years (311 patients), and 5-14 years old (51 patients). However, the proportion of patients positive for *Salmonella* was the highest (9.8%) in this latter age group (5-14 years old).

Although *Salmonella* was the organism of primary interest in this study, other enteric pathogens were also isolated and identified; they were *Vibrio cholerae* O1 (2.2%), *V. cholerae* non-O1 (0.2%), *V. parahaemolyticus* (0.5%), *Shigella* spp (4.2%) and *Campylobacter jejuni* (0.4%). The overall enteric bacterial recovery rate in this study was 14.4%. Mixed isolation of *Salmonella* with other enteric pathogens was seen in which *Salmonella* group B was concomitantly isolated with *S. flexneri* in 0.1%.

Nearly all patients (97%) with non-typhoidal *Salmonella* infection had watery diarrhea. Stool with blood and mucus was not found among patients in this study. A number of patients who were positive for *Salmonella* (70%) were reported to have vomiting. Fever (≥38ºC) was found mainly in patients infected

with *S.* Typhimurium (59%) and *S*. Hadar (68%).

The antimicrobial suscptibility assays of the nontyphoidal *Salmonella* isolates revealed that 5.6% to 66.7% of *Salmonella* isolates showed resistance against 8 antibiotics (Table 2), except for one isolate, *S*. Derby, which was susceptible to all antibiotics. Other *Salmonella* isolates were resistant to two or more antibiotics, namely ampicillin, trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, and nalidixic acid. Ampicillin and tetracycline were two antibiotics to which the majority of *Salmonella* isolates showed resistance. *S.* Typhimurium and *S*. Hadar were resistant to five or more antibiotics with the highest frequency to tetracycline. All isolates were susceptible to norfloxacin, ciprofloxacin and ceftriaxone.

DISCUSSION

Salmonella infection in humans and animals continues to be a distressing health problem worldwide. In many countries the incidence of human nontyphoidal *Salmonella* infection has increased markedly over the years (Olsen *et al*, 2001; Edelstein *et al*, 2004; Kariuki *et a*l, 2006). Multiresistant *Salmonella* isolates of different serovars have become an issue of worldwide concern. Although most nontyphoidal *Salmonella* infections are self-limited, serious sequellae, including systemic infection and death, can occur. Several studies of diarrheal disease have been reported from Indonesia (Subekti *et al*, 1993; Oyofo *et al*, 2002), however, there are few published reports on serovars of *Salmonella* in association with diarrhea in Indonesia.

The most frequently isolated serovars of *Salmonella* from pediatric diarrheal patients in south Jakarta, were *S.* Typhimurium with a prevalence of 2.2%, followed by *S*. Enteritidis with 1.4% (Table 1). The prevalence of *S*. Typhimurium and *S*. Enteritidis as causes of nontyphoidal infection have been documented in many other parts of the world (Center for Disease Control and Prevention, 2000; Voetsch *et al*, 2004) and have been reported to be the two most common serovars isolated in humans (Center for Disease Control and Prevention, 2000). New strains of multi-drug resistant *S*. Typhimurium and *S*. Enteritidis

Table 2
Antibiotic susceptibility of *Salmonella* serovar.

Serovar	Number tested	Number (%) of isolates resistant to antibiotic							
		AM	SXT	C	TE	NA	NOR	CIP	CRO
Typhimurium	18	5 (27.8)	2 (11.1)	4 (22.2)	7 (38.9)	1 (5.6)	0	0	0
Derby	1	0	0	0	0	0	0	0	0
Virchow	6	2 (33.3)	2 (33.3)	0	1 (16.7)	0	0	0	0
Hadar	8	2 (25.0)	1 (12.5)	2 (25.0)	5 (62.5)	1 (12.5)	0	0	0
Newport	3	0	0	0	2 (66.7)	0	0	0	0
Enteritidis	11	3 (27.3)	0	0	0	1 (9.1)	0	0	0
Weltevreden	6	1 (16.7)	0	2 (33.3)	0	0	0	0	0
Lexington	3	0	0	0	1 (33.3)	0	0	0	0

AM - ampicillin
SXT - trimethoprim-sulfamethoxazole
C - chloramphenicol
TE - tetracycline
NA - Nalidixic acid
NOR - norfloxacin
CIP - ciprofloxacin
CRO - ceftriaxone

have emerged in the United States in recent years (Voetsch *et al*, 2004). S. Typhimurium has also been reported to cause outbreaks in Russia and Belarus from 1994 to 2003 with the severity rangins from mild forms of gastroenteritis to life-threatening bacteremia (Edelstein *et al*, 2004). In northern Tanzania, Vaagland *et al* (2004) reported a nosocomial outbreak of neonatal meningitis caused by *S*. Enteritidis and concluded that in the hospital setting *S*. Enteritidis can easily spread by nosocomial transmission, particularly on neonatal wards. The two main serovars which were isolated from cases of bacteremia and gastroenteritis with high fatality in Kenya were S. Typhimurium and S. Enteritidis (Kariuki *et al*, 2006).

Our data show that children aged <5 years comprised the predominant number of diarrheal cases (763 cases) seen in the community health centers, 6.7% (51/763) of them had infection with *Salmonella*. In the age group 5 to 14 years old, we found only 51 diarrheal cases, which is 15 times lower than that found in the age group 1 to 4 years old. However, *Salmonella* was isolated more often in this age group (9.8%). A diarrheal disease survey conducted by Oyofo *et al* (2002) from 1997 to 1999 involving hospitalized cases and outpatients in the community health center reported that greater numbers of patients with positive nontyphoidal *Salmonella* among children < 5 years old were found in the hospitals (3.7%) than in the community health centers (1.1%). The age distribution of cases in their study was similar to our findings, however, the total number of nontyphoidal isolates in our study was greater. From the data reported by Oyofo *et al* (2002), it is probable that infection by nontyphoidal *Salmonella* caused severe disease in children aged < 5 years old, which is shown by the greater number of patients admitted to the hospital.

Salmonellosis is more common in children over 5 years old, where they began to show greater mobility and activities and thus have a greater access to outside foods, such as those sold by food vendors. In 1993, while studying the prevalence of enterotoxigenic *Escherichia coli* (ETEC) in hospitalized pediatric diarrheal cases in Jakarta, Subekti *et al* (1993) reported an isolation rate of 19.1% for nontyphoidal *Salmonella* which was the highest among the reported studies on salmonellosis in Indonesia. It is not clear why the isolation rate of nontyphoidal *Salmonella* has remained low since then.

It was reported that the incidence of salmonellosis was highest during the rainy season in tropical climates and during the warm months in the summer and fall in temperate climates. This coincides with the peak in foodborne outbreaks (Bean *et al*, 1990). A similar observation was described by Graham *et al* (2000) in children with nontyphoidal *Salmonella* infection in Africa which was common during rainy season. However, according to Vaagland *et al* (2004) there was a considerable overlap between those medical conditions since their outbreak investigation was conducted during the dry season. However, according to Oyofo *et al* (2002) there was no association between season and clinical admissions for *Salmonella*-associated diarrhea in Indonesia. We isolated nontyphoidal *Salmonella* all year round during our two-year study.

Although most nontyphoidal *Salmonella* infections are limited to the gastrointestinal tract, invasion of the bloodstream can occur especially in infants and young children. Graham *et al* (2000) reported that in Africa, nontyphoidal *Salmonellae* infections are the most common cause of bloodstream infections in children younger than five years. Multiple outbreaks of nosocomial salmonellosis in Russia and Belarus were reported affecting mostly children younger than a year of age and caused by *S.* Typhimurium. The severity of the disease varied from gastroenteritis to life-threatening bacteremia (Edelstein *et al*, 2004).

Limited data from Indonesia on the incidence of *Salmonella* bacteremia in infants and children younger than a year of age shows that *Salmonella* gastroenteritis preceded bacteremia, *Salmonella* Group C, was usually the cause of the disease (Komalarini *et al*, 1980). *Salmonella* gastroenteritis proved to be a risk of blood stream infection, particularly in infants and young children. Due to difficulties in obtaining blood specimens from infants and young children, we did not do blood cultures in this study.

There is a disturbing general trend in *Salmonella* serovars being resistant to commonly used antimicrobials. Antimicrobial resistance among human *Salmonella* isolates is increasing worldwide and is likely due to the widespread use of antimicrobial agents for the empiric treatment of febrile syndromes and as growth enhancers in animal production (Tollefson *et al*, 1997). High rates of resistance to ampicillin, chloramphenicol, tetracycline, and trimethoprim-sulfamethoxazole have been reported from many areas of the world (Su *et al*, 2004). Also resistance of *Salmonella* to newer antibiotics (quinolones and extended-spectrum cephalosporins) has been demonstrated in developing as well as developed countries with increasing frequency (Frost *et al*, 1996; Herikstad *et al*, 1997; Tauxe, 1997; Su *et al*, 2004).

Our data show that of all serovars isolated *S*. Typhimurium and *S.* Hadar were resistant to 5 out of 8 antibiotics tested ranging from 5.6% to 62.5% (Table 2). However, the majority of isolates were still susceptible to quinolones and ceftriaxone, which suggests that for the present, these antibiotics may still be the drugs of choice for the treatment of nontyphoidal salmonellosis. In view of increasing antimicrobial resistance of *Salmonella* serovars and the impact which it has on public health and economics around the world, continued surveillance of *Salmonella* infections is prudent.

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