Etiologic agents of acute diarrhea in sentinel surveillance sites in Vientiane Capital, Lao People's Democratic
 Republic, 2012-2015

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21 Abstract

- Purpose: This study aims to identify the pathogens of diarrhea in Vientiane Capital, Lao People's DemocraticRepublic (Lao PDR).
- Methods: The data of 2,482 patients who visited eight health facilities due to diarrhea in 2012-2015 were retrospectively reviewed. Stool or rectal swabs collected from all patients were tested for bacteria. Children who were under five years old were additionally tested for rotavirus.
- 27 Results: Of 2,482 cases, 1,566 cases were under five years old and at least one entero-pathogen was detected in 28 475 cases (19.1%). Salmonella species was the most commonly detected bacterial pathogen. Enteropathogenic 29 Escherichia. coli (EPEC) and Salmonella species was the major pathogen in the dry season and the wet season, 30 respectively. Eighty-seven patients tested positive for multiple bacteria. Rotavirus was detected in 291 children 31 under five years old (32.4%), mostly from October to April. The major bacteria of coinfection with rotavirus was 32 EPEC followed by Salmonella species. 33 Conclusion: Salmonella species was the predominant bacterial pathogen of diarrhea of all ages and rotavirus was 34 the predominant pathogen among children under five years old. Further studies examining other types of 35 pathogens for diarrhea and the introduction of a rotavirus vaccine for children are needed in Lao PDR. 36
- 37 Keywords: acute diarrhea; bacteria; etiologic pathogen; Lao PDR; rotavirus; Vientiane Capital.
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40 Introduction

41 Diarrheal diseases account for one in ten child deaths during the first five years of life worldwide [1]. It is 42 estimated that there are 800,000 fatalities by diarrhea globally every year, and the majority of diarrhea cases exist 43 in South Asia and sub-Saharan Africa [2]. Diarrhea can occur from a variety of causes, but the most common 44 causes are the consumption of food or drinks that are contaminated with pathogens such as bacteria, viruses, and 45 parasitic organisms or toxins. Methods for prophylaxis of diarrhea, such as sanitation, safe drinking water, and 46 basic hygiene practice have been improved. However, investigating the etiologic agents would help to understand 47 the overall burden of pathogen-specific diarrheal disease [3]. The transmission of pathogens from person to person 48 is also possible through poor hand hygiene practices [4].

49 Lao People's Democratic Republic (Lao PDR) is a landlocked country located in Southeast Asia, sharing borders 50 with China, Myanmar, Vietnam, Cambodia, and Thailand. Approximately 6.5 million people live within an area 51 of 236,800 m² with a population density of 27.4/km² [5]. According to the health profile statistics by World Health 52 Organization (WHO), diarrhea accounted for 11% of deaths of Lao children below five years old in 2013 [6]. Few 53 studies have examined the etiologic agents of diarrheal diseases in Lao PDR. Two studies were conducted on 54 diarrheal outpatients in hospitals in Vientiane Capital. The main etiologic agents were rotavirus (22.0%), 55 enteropathogenic Escherichia. coli (EPEC, 21.5%), and Shigella flexneri (4.7%) in 1994 and 1995 [7]. 56 Enterotoxigenic Escherichia coli heat-stable enterotoxin (ETEC. ST, 17.2%), Shigella species (spp.) (16.8%), and 57 serogroup-based EPEC (11.0%) were the main etiologic agents in 1996 and 1997 [8]. In another studies conducted 58 at Mahosot Hospital, rotavirus was found to be the main cause of diarrheal diseases in children below five years 59 old [9, 10].

In 2012, the Ministry of Health started the surveillance of the causative etiologies of acute diarrhea in Lao PDR, and eight diarrhea sentinel surveillance sites were established. The aims of the collaborating work were to describe the etiology of diarrhea and bacteremia in patients presenting to health facilities, to enhance the capacity of bacteriology staff for detection and isolation causative factors of diarrhea, to establish a diarrheal surveillance network between key central hospitals in Lao PDR, and to survey antimicrobial resistance of bacteria. This study aimed to report the information regarding etiologic causes of diarrhea in Vientiane Capital by secondary analysis of the laboratory surveillance data from 2012 to 2015.

67

68 Materials and methods

69 Data collection

70 The definition of diarrhea was passing of loose stools three or more times within 24 hours [11]. In the surveillance 71 of the causative etiologies of acute diarrhea, the bacteriology unit of the National Center for Laboratory and 72 Epidemiology (NCLE) collects socio-clinical data and examines stool or rectal swabs from all patients with 73 diarrhea who visited eight diarrhea sentinel surveillance sites in Vientiane Capital (Pediatric Hospital, Sethathirath 74 Hospital, Mahosot Hospital, Mittaphab Hospital, Sisattanak District Hospital, Hadtxayfong District Hospital, 75 Sikhottabong District Hospital, and Ban-hom Health Center). Swabs that were more than 8 days old were not 76 examined. The surveillance was approved as the joint work of the Ministry of Health with WHO by the Ministry 77 of Health (approved activity number 1.44.19.1). This study included patients who visited the sentinel sites from 78 January 2012 to December 2015. Patients who had possibly taken antibiotics before swab tests were not excluded.

79

80 Examinations for bacteria and rotavirus

81 All specimens were tested for Salmonella spp., Shigella spp., Vibrio cholera, Vibrio parahaemolyticus, 82 Aeromonas spp., Plesiomonas shigelloides, Campylobacter spp., EPEC, enteroinvasive Escherichia coli (EIEC), 83 enterotoxigenic Escherichia coli heat-labile enterotoxin (ETEC. LT), ETEC. ST and enterohemorrhagic 84 Escherichia coli (EHEC). Only specimens of patients who were under five years old were tested for rotavirus, 85 due to financial constraints. SD Bioline Rota/Adeno rapid (Standard Diagnostic INC., Gyeonggi-do, Republic of Korea) and RIDASCREEN Rotavirus (R-Biopharm AG, Darmstadt, Germany) were used in 2012. SD Bioline 86 87 Rota/Adeno rapid was used in 2013, and SD Bioline Rota/Adeno rapid and ProSpecT Rotavirus (Thermo Fisher 88 Scientific Inc., Hampshire, UK) were used in 2014 and 2015. The total number of tested samples was 913, 89 including 752 in the dry season (from November to April) and 161 in the wet season (from May to October). However, the results of 14 samples were unable to read. 90

91

92 Polymerase chain reaction for identification of Escherichia coli

When specimens were negative for *Vibrio* spp., *Salmonella* spp., and *Shigella* spp. and positive for *Escherichia coli* (*E. coli*), polymerase chain reaction (PCR) was performed to determine the virulence of pathogenic *E. coli*.
All primer sequences and PCR conditions were developed and modified by the Department of Bacteriology, the
National Institute of Infectious Diseases in Japan. Two working primer mixes, MIX-DEC A and MIX-DEC B,
were used to pathotype *E.coli* (Table 1) [12-14].

98

99 Results

100 Specimens of stool/rectal swabs from 2,482 patients with diarrhea were analyzed. The age ranged from one month 101 to 98 years old and the median age was 1.9 years old. Most patients were younger than 5 years old (n=1,566, 102 63.1%), and patients <1 year old accounted for 643 cases (26.0%) (Table 2). The percentage of patients who 103 visited central hospitals was highest in the age group <1 year old followed by the group of 1-<5 years old. The 104 percentage of males was 52.1% in all cases and higher than that of females in patients <5 years old. The dry season 105 had more diarrheal patients compared to the wet season. The number of diarrhea cases of all ages and <5 years 106 old peaked in February and the second highest peak was from October to November (Fig. 1A). Of the total 107 specimens for bacteria identification, 475 specimens (19.1%) were positive for bacteria, including 354 cases 108 (14.3%) of single bacterial pathogens, 87 cases (3.5%) of multiple bacterial pathogens, and 34 cases (1.4%) of 109 coinfection of bacteria and rotavirus (Table 2). Bacteria-positive cases were consistently observed every month 110 and the positive rate for bacteria ranged from 11.0% to 24.0% (Fig. 1A).

111 The number of cases whose specimens were positive for a single bacterial pathogen was higher in the age group 112 \geq 5 years old (173 cases) than in the other two groups; 66 cases in the group of <1 year and 115 cases in the group 113 of 1–<5 years (Table 2). The results of single bacterial pathogens showed that *Salmonella* spp. was predominantly 114 found (26.3%) followed by EPEC (18.1%) and *Aeromonas* spp. (16.7%) (Table 3). The major pathogen was 115 *Salmonella* spp. in children <1 year, EPEC in children aged 1-<5 years old, and *Aeromonas* spp. in patients \geq 5 116 years old. *Salmonella* spp. and EPEC were the two major pathogens among children <5 years old.

117 Of 354 cases of single bacterial infection, 211 cases occurred in the dry season (Table 4). In the dry season, EPEC

118 was the major pathogen (25.1%) followed by *Salmonella* spp. (23.7%) and *Aeromonas* spp. (14.7%). In contrast,

the major pathogen in the wet season was *Salmonella* ssp. (30.1%). *Plesiomonas shigelloides* and *Vibrio parahaemolyticus* were detected more frequently in the wet season than in the dry season.

121 Multiple bacterial entero-pathogens were found in 87 cases and the predominant age group was the group ≥ 5 years

122 old (45 cases). The major pathogens in mixed bacterial infections were ETEC. LT (31 cases), *Plesiomonas*

shigelloides (24 cases), EPEC (23 cases), and Salmonella spp. (23 cases). Coinfection with EPEC and ETEC. LT

was the most common combination that was found in 10 patients (Table 5).

125 Of specimens from 1,566 children who were under 5 years old, 899 samples were available for the results of

rotavirus test, including 740 of 1,127 specimens (65.7%) in the dry season and 159 of 492 specimens (32.3%) in

the wet season. The specimens of 291 cases (32.4%) were positive for rotavirus. The positive rate for rotavirus
was 39.8% (289 cases) and 1.2% (2 cases) in the dry season and the wet season, respectively. The highest positive
rate was found in cases which occurred in January (53.8%) and rotavirus was not detected from June to September
(Fig. 1B). Coinfections with bacteria and rotavirus were found in 34 cases including 12 cases under one year old
and 22 cases aged 1-<5 years old. The major bacteria of coinfections with rotavirus was EPEC (12 cases) followed
by *Salmonella* spp. (8 cases) (Table 6).

133

134 Discussion

135 In this study, bacterial pathogens were identified in 475 of 2,482 samples, with a positive detection rate of 19.1%. 136 This was lower than the detection rates in most previous studies that were conducted with patients of diarrhea in 137 Asian and African countries and the rate was 20.1-44.2% [15-18]. This study included specimens that were 138 transferred to the laboratory within 7 days after collection due to the limited budget and human resources. 139 According to the practice guidelines for management of infectious diarrhea, the lower detection rate in this study 140 might be because of problems associated with the testing procedures, such as inoculation within 2 hours, storage 141 in a refrigerator if transportation was delayed, agar plates, enrichment broth, technical expertise, as well as the 142 awareness of physicians in terms of diarrheal diagnosis [19, 20].

143 Salmonella spp. was the predominant bacterial cause of diarrhea among all patients. This result was the same as 144 those of previous studies that examined pathogens among not only children aged under five years old, but also 145 people of various ages worldwide [3, 18, 21]. The systematic review including 22 papers reported that Salmonella 146 spp. was the major pathogen of outpatients with diarrhea who were five years old or older [3]. This study also 147 found that Salmonella spp. was the major cause of diarrhea in the wet season. This result was consistent with that 148 of two previous studies conducted in Senegal and Ho Chi Minh City [22, 23]. It was suggested that a higher 149 temperature might increase the number of salmonellosis notifications through an increase of bacterial reproduction 150 at various points along the food chain [24, 25], although there was no association between the prevalence of 151 infections by Salmonella spp. and the average monthly rainfall [23]. However, this may have resulted because the 152 studies were conducted in developed countries, such as Australia and the UK. It is still unexplained in developing 153 countries including Lao PDR.

154 The positive rate for rotavirus among children aged under five years old was 32.4% and it was lower than those155 of the two studies at Mahosot Hospital in Vientiane Capital (53.9-55.9%). It may be because the previous studies

156 included only patients who were admitted to the central hospital due to diarrhea [9, 10]. Immunization for rotavirus 157 has not been introduced in Lao PDR [26], although WHO recommends to include rotavirus vaccines in all national 158 immunization programs [27]. Rotavirus infection was reported to be the predominant cause of diarrheal cases, 159 especially in developing countries, and caused more than half of diarrheal deaths in children under five years old 160 worldwide [28, 29]. This study showed a higher number of rotavirus infections in the dry season the same as the 161 study in 2005-2007. However, this study did not test 41.7% of children under five years old. It was reported that 162 the seasonality of rotavirus was less distinct in tropical countries and in low- and low-middle income countries 163 [30, 11, 31, 32]. These results suggest that children who did not visit health facilities or lived in other provinces 164 might have rotavirus infections with the higher incidence than this study. Rotavirus vaccine should be introduced 165 as part of the national immunization program to protect young children from severe diarrhea in Lao PDR.

Coinfection of bacteria and rotavirus was found in 34 children under five years old (3.9%), but the number of coinfections could be more if all specimens were tested for rotavirus. The major bacteria of coinfection with rotavirus was *E. coli*, especially EPEC. This result was consistent with that of the previous study that was conducted in Vietnam [31]. This study did not examine the association between symptoms and pathogens. Tokoeda et al. reported that coinfections of rotavirus and bacteria caused diarrhea for a prolonged period and severe dehydration [33]. Coinfections are difficult for physicians to diagnose and treat, and therefore the results of this study may be useful for physicians to understand about coinfections and causes of diarrhea.

173 There are some limitations to this study. First, the results of etiologic agents in this study may not be representative 174 of the entire country because all sentinel sites were located in Vientiane Capital. However, most health facilities 175 in rural areas cannot perform laboratory tests on patients with diarrhea, and the culture and climate are similar 176 throughout the country. Therefore, the results of this study could be considered useful for medical professionals 177 to understand the causes of diarrhea to provide treatment to patients in Lao PDR. Secondly, this study did not 178 show the reason why the detection rate of bacteria or rotavirus was lower than that observed in previous studies 179 in Lao PDR or other countries. It is possible that some bacterial pathogens were not detected owing to swabs with 180 inadequate volume of stool or self-medication with antibiotics. In this study, 104 patients had taken antibiotics 181 before the examinations. In Lao PDR, antibiotics can be obtained at pharmacies without a prescription, if the 104 182 patients were excluded, the detection rate of pathogens might be higher in this study. Thirdly, 653 samples were 183 not tested for rotavirus due to the limitation of financial support. We were unable to increase the possibility of 184 rotavirus detection, as well as assess the true seasonality of rotavirus and coinfection of bacteria and rotavirus.

185 Stool specimens should be tested for rotavirus throughout the year in order to show the true seasonality of rotavirus186 as well as other types of pathogens, such as norovirus and protozoa, including giardia and amoeba.

187 In conclusion, this study showed that bacterial pathogens were identified in 19.1% of patients with diarrhea of all

ages and rotavirus was detected in 32.4% of children <5 years old in Vientiane Capital. Salmonella spp. was the

189 predominant bacterial pathogen in single bacterial infections of all ages. Rotavirus was the predominant pathogen

190 of diarrhea among children <5 years old. The numbers of diarrheal patients and rotavirus infections were higher

in the dry season than the wet season. Rotavirus vaccine should be included in the national immunization program

to prevent children's health in Lao PDR. Further studies examining other types of pathogens for diarrhea in the

same and other provinces in Lao PDR will be needed in the future.

194

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200 Contributors

Souphatsone Houattongkham, Eiko Yamamoto and Onechanh Keosavanh designed the study. Souphatsone Houattongkham, Noikaseumsy Sithivong, and Arounnapha Vongduangchanh collected the data. ouphatsone Houattongkham, Eiko Yamamoto and Souphalak Inthapatha carried out the analysis. Yu Mon Saw, Tetsuyoshi Kariya and Nobuyuki Hamajima provided the epidemiological advice. Souphatsone Houattongkham wrote the first draft and Eiko Yamamoto and Nobuyuki Hamajima revised the manuscript. All authors reviewed the manuscript and approved the final version of manuscript.

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208 Compliance with ethical standards

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213

214 Conflict of interest

215 The authors declare that there are no conflicts of interest.

216

217 Ethical approval

- 218 The study did not require an ethical approval.
- 219
- 220 Informed consent
- 221 The study did not require an informed consent.

222 Figure legends

223 Fig. 1 Numbers of patients with diarrhea caused by bacterial, rotavirus, and coinfections from 2012 to 2015 224 according to the month of onset. The dry season is from November to April and the wet season is from May to 225 October. A, Number of total patients and patients under five years old, and the number and the rate of patients 226 whose specimens were positive for bacteria. B, Number of patients under five years old and the patients whose 227 results of rotavirus were available, and the number and the rate of patients whose specimens were positive for 228 rotavirus. The results of 899 cases were available, although 913 cases were tested but in 14 cases we were unable 229 to read the results. Grey bold line, number of all-aged patients; black bold line, number of patients <5 years old; 230 black thin line, number of patients <5 years old who tested for rotavirus, black line with black circle, number of 231 positive cases for bacteria; black dot line with white circle, positive rate of bacteria, black line with black square, 232 number of positive cases for rotavirus; black dot line with white square, positive rate of rotavirus.

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Gene	Orientation	Primer sequence $(5^{\prime} \rightarrow 3^{\prime})$	Working primer mix
eaeA-new	F	AACGGCTATTTCCGCATGAG	
eaeA-new	R	CACATAAGCMGGCAAAATAGCCTG	
bfpB	F	GACACCTCATTGCTGAAGTCG	
bfpB	R	GCCCAGAACACCTCCGTTAT	MIX DEC A
stx1	F	TATCTGGATTTAATGTCGCATAG	MIX DEC A
stx1	R	AGAACGCCCACTGAGATCATC	
mMK2	F	GAGTTTACGATAGACCTTTCGAC	
mMK2	R	GGCCACATATAAATTATTTTGCTC	
ST1a	F	GCAATTTTTATTTCTGTATTATCTT	
St1a	R	GGATTACAACAAAGTTCACAG	
ST1b	F	TTTATTTTTCTTTCTGTATTGTCTT	
St1b	R	GGATTACAACACAATTCACAG	MIX DEC B
lt	F	CTATTACAGAACTATGTTCGGAATATC	MIX DEC B
lt	R	GGGGGTTTTATTATTCCATACAC	
ipaH	F	GTTCCTTGACCGCCTTTCCGATACCGTC	
ipaH	R	GCCGGTCAGCCACCCTCTGAGRGTAC	

Table 1 Primers for subgroups of Escherichia coli

		То	tol					
	<		1			5	(N=2	
-	(N=	,	(N=9	,	· · · ·	916)		
	n	%	n	%	n	%	Ν	%
Sex								
Male	381	59.3	523	56.7	388	42.4	1,292	52.1
Female	262	40.7	400	43.3	528	57.6	1,190	47.9
Health facility ^a								
Central hospital	480	74.7	581	62.9	449	49.0	1,510	60.8
District hospital	108	16.8	240	26.0	317	34.6	665	26.8
Health center	55	8.6	102	11.1	150	16.4	307	12.4
Season ^b								
Dry season	434	67.5	693	69.2	468	51.1	1541	62.1
Wet season	209	32.5	284	30.8	448	48.9	941	37.9
Bacterial entero-pathogen								
Negative	551	85.7	758	82.1	698	76.3	2,007	80.9
Single bacteria	66	10.3	115	12.5	173	18.9	354	14.3
Multiple bacteria	14	2.2	28	3.0	45	4.9	87	3.5
Bacteria + rotavirus	12	1.9	22	2.4	-	-	34	1.4
Rotavirus								
Negative	256	68.6	352	66.9	-	-	608	67.6
Positive	117	31.4	174	33.1	-	-	291	32.4
Equivocal	6	-	8	-	-	-	14	-
Not tested	264	-	389	-	916	-	1,569	-

Table 2 Characteristics of patients with diarrhea

^aCentral hospital includes Pediatric Hospital, Sethathirath Hospital, Mahosot Hospital and Mittaphab Hospital, District hospital includes Sisattanak District Hospital, Hadtxayfong District Hospital and Sikhottabong District Hospital, and health center is Ban-hom Health Center. ^bThe dry season is from November to April and the wet season is from May to October.

			T. 64.01						
Entero-pathogen		<1 I=66)		-<5 =115)		≥5 =173)	Total (N=354)		
	n	%	n	%	n	%	Ν	%	
Salmonella spp.	34	51.5	23	20.0	36	20.8	93	26.3	
EPEC	15	22.7	29	25.2	20	11.6	64	18.1	
EHEC	5	7.6	5	4.3	7	4.0	17	4.8	
EIEC	3	4.5	10	8.7	2	1.2	15	4.2	
ETEC. LT	1	1.5	11	9.6	2	1.2	14	4	
ETEC. ST	1	1.5	9	7.8	3	1.7	13	3.7	
Aeromonas spp.	4	6.1	9	7.8	46	26.6	59	16.7	
Shigella spp.	1	1.5	16	13.9	18	10.4	35	9.9	
Plesiomonas shigelloides	1	1.5	3	2.6	25	14.5	29	8.2	
Vibrio parahaemolyticus	0	0.0	0	0.0	11	6.4	11	3.1	
Vibrio cholera non 01/0139	0	0.0	0	0.0	3	1.7	3	0.8	
<i>Campylobacter</i> spp.	1	1.5	0	0.0	0	0.0	1	0.3	

Table 3 Single bacterial entero-pathogen of diarrhea in age groups

spp., species; EPEC, Enteropathogenic *Escherichia coli*; EHEC, Enterohemorrhagic *Escherichia coli*; EIEC, Enteroinvasive *Escherichia coli*; ETEC. LT, Enterotoxigenic *Escherichia coli* heat-labile enterotoxin; ETEC. ST, Enterotoxigenic *Escherichia coli* heat-stable enterotoxin.

Table 4 Seasonal variation of single bacterial entero-pathogen of diarrhea

Entoro pothogons	Dry	season	Wet s	season	Total		
Entero-pathogens	n	%	n	%	N	%	
Salmonella spp.	50	23.7	43	30.1	93	26.3	
EPEC	53	25.1	11	7.7	64	18.1	
EHEC	16	7.6	1	0.7	17	4.8	
EIEC	10	4.7	5	3.5	15	4.2	
ETEC. LT	12	5.7	2	1.4	14	4.0	
ETEC. ST	9	4.3	4	2.8	13	3.7	
Aeromonas spp.	31	14.7	28	19.6	59	16.7	
<i>Shigella</i> spp.	22	10.4	13	9.1	35	9.9	
Plesiomonas shigelloides	4	1.9	25	17.5	29	8.2	
Vibrio parahaemolyticus	2	0.9	9	6.3	11	3.1	
Vibrio cholera non 01/0139	1	0.5	2	1.3	3	0.8	
Campylobacter spp.	1	0.5	0	0.0	1	0.2	
Total	211	100.0	143	100.0	354	100.0	

The dry season is from November to April and the wet season is from May to October. spp., species; EPEC, Enteropathogenic *Escherichia coli*; EHEC, Enterohemorrhagic *Escherichia coli*; EIEC, Enteroinvasive *Escherichia coli*; ETEC. LT, Enterotoxigenic *Escherichia coli* heatlabile enterotoxin; ETEC. ST, Enterotoxigenic *Escherichia coli* heat-stable enterotoxin.

Table 5 Multiple bacterial pathogens of 87 patients with diarrhea

	Salmonella spp.	EPEC	EHEC	EIEC	ETEC. LT	ETEC. ST	Aeromonas spp.	Shigella spp.	Plesiomonas shigelloides	Vibrio parahaemolyticus	Campylobacter spp.	EPEC + ETEC.LT	EPEC + ETEC.ST	EHEC + ETEC. LT	EHEC + ETEC. ST	EHEC + Salmonella spp.	ETEC. LT + ETEC. ST	ETEC. ST + Salmonella spp.	Salmonella spp. + Plesiomonas shigelloides	Salmonella spp. + Vibrio parahaemolyticus	Plesiomonas shigelloides + Vibrio parahaemolyticus	Total
Salmonella spp.	-	4	1	0	2	1	5	1	5	3	0	0	0	0	0	0	0	0	0	0	1	23
EPEC	4	-	0	1	10	4	1	0	1	0	1	0	0	0	0	0	1	0	0	0	0	23
EHEC	1	0	-	2	5	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	1	12
EIEC	0	1	2	-	0	4	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	8
ETEC. LT	2	10	5	0	-	7	2	0	3	0	0	0	1	0	1	0	0	0	0	0	0	31
ETEC. ST	1	4	0	4	7	-	0	0	0	0	0	1	0	1	0	1	0	0	0	0	0	19
Aeromonas spp.	5	1	0	0	2	0	-	3	5	3	0	0	0	0	0	0	0	0	0	0	0	19
Shigella spp.	1	0	0	0	0	0	3	-	3	0	0	0	0	0	0	0	0	0	0	0	0	7
Plesiomonas shigelloides	5	1	1	1	3	0	5	3	-	4	0	0	0	0	0	0	0	0	0	1	0	24
Vibrio parahaemolyticus	3	0	0	0	0	0	3	0	4	-	0	0	0	0	0	0	0	0	1	0	0	11
Campylobacter spp.	0	1	0	0	0	0	0	0	0	0	-	0	0	0	0	0	0	0	0	0	0	1

spp., species; EPEC, Enteropathogenic *Escherichia coli*; EHEC, Enterohemorrhagic *Escherichia coli*; EIEC, Enteroinvasive *Escherichia coli*; ETEC.LT, Enterotoxigenic *Escherichia coli* heat-labile enterotoxin; ETEC. ST, Enterotoxigenic *Escherichia coli* heat-stable enterotoxin.

	Age	- Total	
Bacteria (+ rotavirus)	<1	1-<5	
	(N=12)	(N=22)	(N=34)
EPEC	4	8	12
EIEC	0	3	3
ETEC.LT	0	3	3
EHEC	0	1	1
EPEC + ETEC.LT	1	0	1
Salmonella spp.	5	3	8
Aeromonas spp.	1	1	2
<i>Shigella</i> spp.	0	2	2
Aeromonas spp. + Salmonella spp.	1	0	1
<i>Campylobacter</i> spp.	0	1	1

Table 6 Bacteria of coinfections with rotavirus among children under five years old

EPEC, Enteropathogenic *Escherichia coli;* EIEC, Enteroinvasive *Escherichia coli;* ETEC. LT, Enterotoxigenic *Escherichia coli* heat-labile enterotoxin; EHEC, Enterohemorrhagic *Escherichia coli;* spp., species.

