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RESEARCH ARTICLE

Enterobacteriaceae Responsible For Acute Gastroenteritis in Children and Their Resistance to Systemic Antibiotics

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ABSTRACT

AMR is recognized as a global threat to human health; however, information is limited in children with EDA in rural high Andean areas. The purpose of this study was to evaluate the presence of enterobacteria causing acute gastroenteritis in children and their resistance to systemic antibiotics. A total of 314 fecal samples from children aged 1 to 5 years from 7 health micro-networks in Huancavelica, Perú were analyzed. The samples were cultured on selective agar to detect Escherichia coli, *Salmonella spp*, *Shigella spp*, confirmed by macro-microscopic characterization and biochemical tests. Antimicrobial susceptibility (four commonly used antibiotics) was determined by the Kirby-Bauer method. Strains of *E. coli* (47%), *Salmonella spp* (28%) and Shigella spp (26%), with high frequencies of these three bacteria being observed in children from micro-networks of Ascensión (55-44-50%), Izcuchaca (70-81-83%) and Health Centers of Huachocolpa (85-70-75%), Pucaccocha (71-57-35%), Izcuchaca (65-82-60%) and Pallalla (100-57-80%), with girls aged 3 to 5 years being more susceptible to EDA. Resistance to oxytetracycline, chloramphenicol, sulfamethoxazole-trimethropin, ranged from 48-97% and ceftriaxone was sensitive (40-51-53%) for the three bacteria, showing similar resistance and sensitivity behaviors among micro-networks, health centers and in three-year-old girls. Stool cultures from children with acute gastroenteritis from various rural hospitals in Huancavelica, Peru, confirm the predominant presence of multidrug-resistant E. coli, Salmonella spp., and Shigella spp.

INTRODUCTION

Acute Diarrheal Disease (EDA) in infants under 5 years of age is an emerging enteric pathology that leads to high mortality rates, ranging from 0.8 to 2 million deaths among children globally (Alzamora et al., 2019; Dyar et al., 2012). It is estimated that one billion episodes of diarrhea occur annually in children worldwide (Bryce et al., 2016; Hertz et al., 2016), with the highest incidence reported in Latin America, Africa, Central America (Dyar et al., 2012; Birgy et al., 2020), and Southeast Asia (Valenza et al., 2019; Birgy et al., 2020). The morbidity rate for acute diarrheal disease is considerably high, at 45% (Alzamora et al., 2019; Bryce et al., 2016). As a result, these infections rank second among infectious processes, with the primary causative agents being diarrheagenic *E. coli*, *Salmonella spp*., and Shigella spp. of the Enterobacteriales order (Hertz et al., 2016; Harada et al., 2021). However, there are few reports on the etiology and epidemiology of these bacterial agents in high-Andean rural populations (Vélez et al., 2015).

The excessive and inappropriate use of antimicrobials in EDA is a common practice (Olaiz-Fernández et al., 2020; Servicio Nacional de Meteorología e Hidrología [SENAMHI], 2017). Consequently, antimicrobial resistance has necessitated changes in therapeutic management in hospital settings, which has reduced the availability of first-choice drugs and posed a challenge for healthcare personnel and an economic burden for Latin American governments (Hertz et al., 2016; Soliman et al., 2020).

The World Health Organization (WHO) and the National Plan against Antibiotic Resistance (PRAN) argue that it is essential to have sensitivity profiles for adequate and timely treatment (Alzamora et al., 2019; Hertz et al., 2016). Many surveillance programs aim to address antimicrobial resistance (RAM) profiles (Alzamora et al., 2019; Daniel, 2007) and the prevalence of multidrug-resistant (MDR) organisms in patients with EDA (Olaiz-Fernández et al., 2020; Daniel, 2007). However, few studies assess RAM rates and MDR distributions in children from rural high-Andean hospitals (De Kraker et al., 2016; SENAMHI, 2017). In this study, we present a primary report on the etiology and resistance profiles of the primary pathogens in children with EDA, providing useful guidance for anti-infective therapy in pediatric patients at the local and regional levels.

The purpose of the study was to evaluate the prevalence of Enterobacteriaceae and their antibiotic resistance profiles against first-line antibiotics in children with acute gastroenteritis from the Health Micro-Networks of Huancavelica, Peru, as well as compare these profiles by age group, sex, and origin.

MATERIALS AND METHODS

Materials and Methods

Population and sample: The study considered 314 children under 5 years of age, of both sexes, from 7 Health Micro-Networks in Huancavelica, Peru, as follows: Ascensión (62 children), San Cristóbal (31 children), Santa Ana (34 children), Yauli (36 children), Acoria (53 children), Ayaccocha (55 children), and Izcuchaca (43 children), all located within the jurisdiction of Huancavelica, Peru. A non-probabilistic sampling by census was used to determine the number of children to be sampled (Bergey's, 2008).

Inclusion criteria included children aged 1 to 5 years of both sexes with acute gastroenteritis, as recorded in the general epidemiological records of the Regional Health Directorate of Huancavelica (Diresa), Peru, after obtaining informed consent from the child's representative. Additionally, children who had not received any antidiarrheal treatment in the four months prior to the study were included.

Technical information: The studies were conducted in 7 Health Micro-Networks of Huancavelica-Peru (Ascensión, San Cristóbal, Santa Ana, Yauli, Acoria, Ayaccocha, Izcuchaca) and their respective Health Centers, located geographically between 25 to 45 kilometers northwest, southeast, and northeast of Huancavelica province, at altitudes between 3,720 and 4,200 meters above sea level, with annual temperatures ranging between 18°C and -5°C (Daniel, 2007). The field (sampling) and laboratory studies were conducted between January and April 2024.

Data collection: A total of 314 stool samples (5 g) were independently collected from the children included in the study through rectal swabs in cryovials with 0.1% Peptone buffer (5 mL) during the morning (6:00 am to 7:30 am) at the Health Micro-Networks of Huancavelica-Peru, following strict biosecurity measures. All collected samples were labeled (origin, date, altitude, sex, age) and transported in a thermal container (KST Thermo with biological ice) at 8-10°C, within less than 4 hours after collection, to the Animal Health Laboratory, microbiology area of the National University of Huancavelica – Peru, for microbiological analysis.

a) Isolation of enteropathogenic bacteria of the 314 stool samples collected, 5µL were independently taken from each patient and streaked on Xylose Lysine Deoxycholate (XLD) agar. Subsequently, subcultures were made on Bismuth Sulfite agar for *Salmonella spp*., and for *E. coli*, cultured on Eosin Methylene Blue (EMB) agar, followed by subculturing on HiCrome™ *E. coli* Agar. For *Shigella spp*., samples were cultured on Salmonella-Shigella (SS) agar and incubated at 37ºC for 24 hours. Quality control of the

media was performed to rule out opportunistic microorganism growth by verifying physical characteristics (pH, appearance, color, precipitates), sterility, and growth promotion capacity in reference strains, using Escherichia coli NCTC 13353™ Culti-Loops obtained from Thermo Scientific™ (Clinical and Laboratory Standards Institute [CLSI], 2023).

b) Bacterial identification. The confirmation of *Salmonella spp*., E. coli, and *Shigella spp*. strains was performed through macroscopic characterization (shape, color, edge, elevation, and consistency), microscopic examination (group and Gram staining), and biochemical tests such as TSI (Triple Sugar Iron Agar), LIA (Lysine Iron Agar), Simmons Citrate Agar, SIM (Sulfide Indole Motility), Voges-Proskauer, and Catalase tests (CLSI, 2023). The confirmation of Shigella spp. was further supported by serological tests. Suspected strains were cultured on TSA agar and incubated at 37°C for 24 hours, and polyvalent antisera from the four groups (Probac Co.) were used according to the manufacturer's instructions: first, antisera against Shigella flexneri, followed by Shigella sonnei, Shigella boydii, and finally Shigella dysenteriae (Bergey's, 2008). The pathogenicity mechanism of *E. coli* was confirmed using the SHIGA TOXIN QUIK CHEK™ membrane-based rapid enzyme immunoassay (Boone et al., 2016).

c) Antibiotic susceptibility testing

Antibiotic sensitivity was determined using the Kirby-Bauer method. Strains of *Salmonella spp*. (89 strains), *E. coli* (149 strains), and Shigella spp. (83 strains) were independently suspended in saline solution (0.9%) to a turbidity of 0.5 on the McFarland scale and enriched in Brain Heart Infusion broth (BHI) incubated at 37°C for 5 hours. The strains were then cultured on Mueller-Hinton agar plates with the following antibiotics: Oxytetracycline (OTEX=30 µg), Sulfamethoxazole-trimethoprim (TSM=80-400 µg), Chloramphenicol (C=30 µg), and Ceftriaxone (CRO=30 µg), and incubated at 37°C for 24 hours under aerobic conditions. Interpretation of the results was based on the M100-S25 Performance Standards for Antimicrobial Susceptibility Testing manual (CLSI, 2023), and inhibition zones were measured using the Scan® 4000 device (Interscience).

Statistical analysis: A descriptive, prospective, cross-sectional study was conducted to determine the frequency of enteropathogenic bacteria and their antibiotic resistance for each antibiotic, using SPSS version 23.

Bioethical aspects: The parents of the children signed informed consent forms, and authorization was obtained from the community, the Health Micro-Networks of Huancavelica-Peru, and the evaluation committee of the Faculty of Nursing of the National University of Huancavelica, Peru (Resolution No. 075-2020-D-FEN-R-UNH).

RESULTS

A total of 149 (47%) positive cultures for Escherichia coli, 89 (28%) for Salmonella spp., and 83 (26%) for Shigella spp. were isolated from 314 analyzed samples (see Table 1).

Table 1. Enteropathogenic Bacteria in Children with Acute Gastroenteritis in Huancavelica, Peru

Bacterias	N	Positive		Negative	
		F	%	F	%
E. coli	314	149	47,0	165	53,0
Salmonella spp	314	89	28,0	225	72,0
Shigella spp	314	83	26,0	231	74,0

Leyend: N = Number of samples, F = Frequency of positive bacteria.

In relation to the Micro-networks, a high predominance of E. coli, *Salmonella spp*., and Shigella spp. was observed in the Micro-networks of Ascensión (55%, 44%, and 50%), Izcuchaca (70%, 81%, and 83%), and the Health Centers of Huachocolpa (85%, 70%, and 75%), Pucaccocha (71%, 57%, and 35%), Izcuchaca (65%, 82%, and 60%), and Pallalla (100%, 57%, and 80%).

Table 2. ***Enteric bacteria in children with acute gastroenteritis, by origin, age group, and sex (N=314).***

Study groups	N	*E. coli*				*Salmonella spp*				Shigella spp			
		Positive		Negative		Positive		Negative		Positive		Negative	
		F	%	F	%	F	%	F	%	F	%	F	%
Micro-networks													
Ascensión	62	34	54,84	28	45,16	27	43,55	35	56,45	31	50,00	31	50,00
San Cristóbal	31	11	35,48	20	64,52	4	12,90	27	87,10	7	22,58	24	77,42
Ayaccocha	55	18	32,73	37	67,27	23	41,82	32	58,18	11	20,00	44	80,00
Santa Ana	34	11	32,35	23	67,65	9	26,47	25	73,53	3	8,82	31	91,18
Izcuchaca	43	30	69,77	13	30,23	35	81,40	8	18,60	36	83,72	7	16,28
Yauli	36	6	16,67	30	83,33	10	27,78	26	72,22	6	16,67	30	83,33
Acoria	53	33	62,26	20	37,74	10	18,87	43	81,13	19	35,85	34	64,15
Total	314	143	46.0	171	54.0	118	38.0	196	62.0	113	36.0	201	64.0
Health centers													
Ascensión	42	17	40,48	25	59,52	13	30,95	29	69,05	16	38,10	26	61,90
Huachocolpa	20	17	85,00	3	15,00	14	70,00	6	30,00	15	75,00	5	25,00
San Cristóbal	31	11	35,48	20	64,52	4	12,90	27	87,10	7	22,58	24	77,42
Pucaccocha	14	10	71,43	4	28,57	8	57,14	6	42,86	5	35,71	9	64,29
Huanaspampa	20	-	-	20	100,0	3	15,00	17	85,00	-	-	20	100.0
Ayaccocha	21	8	38,10	13	61,90	12	57,14	9	42,86	6	28,57	15	71,43
Santa Ana	34	11	32,35	23	67,65	9	26,47	25	73,53	3	8,82	31	91,18
Izcuchaca	23	15	65,22	8	34,78	19	82,61	4	17,39	14	60,87	9	39,13
Yauli	36	6	16,67	30	83,33	10	27,78	26	72,22	6	16,67	30	83,33
Cochan	15	7	46,67	8	53,33	3	20,00	12	80,00	1	6,67	14	93,33
Acoria	23	11	47,83	12	52,17	3	13,04	20	86,96	10	43,48	13	56,52
Pallalla	35	35	100.0	-	-	20	57,14	11	31,43	28	80,00	7	20,00
Total	314	148	47.0	166	53.0	118	38.0	192	62.0	111	35.0	203	65.0
Ages													
One year	49	11	22,45	38	77,55	15	30,61	34	69,39	14	28,57	35	71,43
Two years	71	20	28,17	51	71,83	19	26,76	52	73,24	20	28,17	51	71,83
Three years	97	65	67,01	32	32,99	53	54,64	44	45,36	46	47,42	51	52,58
Four years	53	26	49,06	27	50,94	17	32,08	36	67,92	20	37,74	33	62,26
Five years	44	28	63,64	16	36,36	18	40,91	26	59,09	13	29,55	31	70,45
Total	314	150	48,0	164	52,0	122	39,0	192	61,0	113	36,0	201	64,0
Sex													
Male	139	57	41,01	82	58,99	38	27,34	101	72,66	39	28,06	100	71,94
Female	175	93	53,14	82	46,86	84	48,00	91	52,00	74	42,29	101	57,71
Total	314	150	48,0	165	52,0	112	37,0	192	63,0	113	36,0	201	64,0

It is worth noting that in the Huanaspampa Health Center, only *Salmonella spp*. was detected (15%). Additionally, E. coli, *Salmonella spp*., and Shigella spp. were more frequently observed in the age group of three-year-olds (67%, 55%, and 47%, respectively), and among females (*E. coli* 53%, *Salmonella spp*. 48%, and Shigella spp. 42%).

From the total of 149 strains of Gram-negative bacteria identified, there were high proportions of *E. coli* resistance to oxytetracycline (97%), chloramphenicol (83%), and sulfamethoxazole-trimethoprim (48%). *Salmonella spp*. showed resistance to oxytetracycline (96%), chloramphenicol (85%), sulfamethoxazole-trimethoprim (56%), and ceftriaxone (57%). Additionally, Shigella spp. exhibited resistance to oxytetracycline (89%), chloramphenicol (57%), and ceftriaxone (42%). However, *E. coli* was found to be sensitive to ceftriaxone and sulfamethoxazole-trimethoprim, as detailed in Table 3.

Table 3. Antibiotic resistance of enteric bacterial strains in children with acute gastroenteritis

Bacteria/antibiotics	N	Sensitive		Intermediate		Resistant	
		F	%	F	%	F	%
E. coli							
OTX	149	5,0	3,0	-	-	145	97,0
CRO	149	76,0	51,0	-	-	44	30,0
TSM	149	42,0	28,0	36	24,0	72	48,0
C	149	20,0	13,0	6	4,0	123	83,0
Salmonella spp							
OTX	89	4	4,0	-	-	85	96,0
CRO	89	36	40,0	2	2,0	51	57,0
TSM	89	20	22,0	19	21,0	50	56,0
C	89	8	9,0	5	6,0	76	85,0
Shigella spp							
OTX	83	8	10,0	1	1,0	74	89,0
CRO	83	44	53,0	4	5,0	35	42,0
TSM	83	40	48,0	31	37,0	12	14,0
C	83	31	37,0	5	6,0	47	57,0

Leyend: OTX = Oxytetracycline, CRO = Ceftriaxone, TSM = Sulfamethoxazole-Trimethoprim, C = Chloramphenicol.

E. coli strains showed high resistance to oxytetracycline (64-100%) and chloramphenicol (64-100%) across all micro-networks and health centers, with similar resistance patterns for ceftriaxone and sulfamethoxazole-trimethoprim in Izcuchaca and Yauli. Resistance was particularly evident in children aged three to five years and in females, with strains resistant to more than two antibiotics.

Salmonella spp. exhibited resistance to oxytetracycline (67-100%) and chloramphenicol (63-100%) across all locations. Ceftriaxone and sulfamethoxazole-trimethoprim also showed resistance in certain micro-networks and health centers, while Shigella spp. strains were resistant to oxytetracycline (83-100%) and chloramphenicol (33-100%) but remained sensitive to ceftriaxone and sulfamethoxazole-trimethoprim in some areas. Resistance was more common in children aged one to three years and females. The age and gender-related resistance patterns were like those observed in the other bacteria studied (Table 6).

Table 4. ***Antibiotic resistance of E. coli isolated from children with acute gastroenteritis, by origin, age group, and sex (N=149).***

Study group	N	OTX						CRO						TSM						C					
		S		I		R		S		I		R		S		I		R		S		I		R	
		F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%
Micro-network																									
Ascensión	33	1	3,0	-	-	32	97,0	25	76,0	-	-	8	24,0	14	42,0	9	27,0	10	30,0	8	24,0	1	3,0	24	73,0
San Cristóbal	11	-	-	-	-	11	100,0	5	45,0	-	-	6	55,0	6	55,0	4	36,0	1	9,0	3	27,0	-	-	8	73,0
Ayaccocha	18	1	6,0	-	-	17	94,0	16	89,0	-	-	2	11,0	11	61,0	5	28,0	2	11,0	4	22,0	-	-	13	72,0
Santa Ana	11	-	-	-	-	11	100,0	6	55,0	-	-	5	45,0	4	36,0	3	27,0	4	36,0	2	18,0	2	18,0	7	64,0
Izcuchaca	37	1	3,0	-	-	36	97,0	2	5,0	-	-	35	95,0	3	8,0	1	3,0	34	92,0	5	14,0	-	-	32	86,0
Yauli	6	1	17,0	-	-	5	83,0	1	17,0	-	-	5	83,0	1	17,0	2	33,0	3	50,0	-	-	-	-	6	100,0
Acoria	33	1	3,0	-	-	32	97,0	21	64,0	-	-	12	36,0	13	39,0	12	36,0	8	24,0	15	45,0	3	9,0	15	45,0
Total	149	5	3,0	-	-	144	97.0	76	51,0	-	-	73	49,0	52	35,0	36	24,0	62	42,0	37	25,0	6	4,0	105	70,0
Health center																									
Ascensión	16	-	-	-	-	16	100,0	15	94.0	-	-	1	6,0	8	50,0	2	13,0	6	38,0	6	38,0	1	6,0	9	56,0
Huachocolpa	17	1	6,0	-	-	16	94,0	10	59,0	-	-	7	41,0	6	35,0	7	41,0	4	24,0	2	12,0	-	-	15	88,0
San Cristobal	11	-	-	-	-	11	100,0	5	45,0	-	-	6	55,0	6	55,0	4	36,0	1	9,0	3	27,0	-	-	8	73,0
Pucaccocha	10	1	10,0	-	-	9	90,0	10	100,0	-	-	-	-	9	90,0	1	10,0	-	-	4	40,0	-	-	6	60,0
Ayaccocha	8	-	-	-	-	8	100,0	6	75,0	-	-	2	25,0	2	25,0	4	50,0	2	25,0	-	-	-	-	7	88,0
Santa Ana	11	-	-	-	-	11	100,0	6	55,0	-	-	5	45,0	4	36,0	3	27,0	4	36,0	2	18,0	2	18,0	7	64,0
Izcuchaca	26	1	4,0	-	-	25	96,0	2	8,0	-	-	24	92,0	3	12,0	1	4,0	22	85,0	5	19,0	-	-	21	81,0
Yauli	6	1	17,0	-	-	5	83.0	1	17,0	-	-	5	83,0	1	17,0	2	33,0	3	50,0	-	-	-	-	6	100,0
Cochan	7	1	14,0	-	-	6	86,0	6	86,0	-	-	1	14,0	5	71,0	1	14,0	1	14,0	-	-	2	29,0	5	71,0
Acoria	11	-	-	-	-	11	100,0	11	100,0	-	-	-	-	2	18,0	3	27,0	6	55,0	9	82,0	-	-	2	18,0
Pallalla	26	-	-	-	-	26	100,0	4	15,0	-	-	22	85,0	6	23,0	20	77,0	1	4,0	2	8,0	1	4,0	23	88,0
Total	149	5	3,0	-	-	144	97,0	76	51,0	-	-	73	49,0	52	35,0	48	32,0	50	34,0	33	22,0	6	4,0	109	73,0
Ages																									
One year	10	1	10,0	-	-	9	90,0	7	70,0	-	-	3	30,0	7	70,0	-	-	3	30,0	2	20,0	-	-	8	80,0
Two years	21	-	-	-	-	21	100,0	13	62,0	-	-	8	38,0	4	19,0	8	38,0	9	43,0	5	24,0	1	5,0	15	71,0
Three years	64	1	2,0	-	-	63	98,0	6	9,0	-	-	58	91,0	17	27,0	47	73,0	7	11,0	57	89,0	3	5,0	61	95,0
Four years	26	-	-	-	-	26	100,0	14	54,0	-	-	12	46,0	15	58,0	9	35,0	2	8,0	7	27,0	1	4,0	17	65,0
Five years	28	3	11,0	-	-	25	89,0	18	64,0	-	-	10	36,0	9	32,0	8	29,0	11	39,0	9	32,0	1	4,0	18	64,0
Total	149	5	3,0	-	-	144	97,0	58	39,0	-	-	91	61,0	52	35,0	72	48,0	32	21,0	80	54,0	6	4,0	119	80,0
Sex																									
Male	56	4	7,0	-	-	52	93,0	34	61,0	-	-	22	39,0	23	41,0	18	32,0	15	27,0	15	27,0	3	5,0	37	66,0
Female	93	1	1,0	-	-	92	99,0	42	45,0	-	-	51	55,0	29	31,0	18	19,0	46	49,0	22	24,0	3	3,0	68	73,0
Total	149	5	3,0	-	-	144	97,0	76	51,0	-	-	73	49,0	52	35,0	36	24,0	61	41,0	37	25,0	6	4,0	105	70.0

Legend: Oxytetracycline (OTEX), Sulfamethoxazole-trimethoprim (TSM), Chloramphenicol (C), Ceftriaxone (CRO).

Table 5. ***Antibiotic resistance of Salmonella spp. isolated from children with acute gastroenteritis, by origin, age group, and sex (N=89).***

Study group	N	OTX						CRO						TSM						C					
		S		I		R		S		I		R		S		I		R		S		I		R	
		F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%
Micro-network																									
Ascensión	27	1	4,0	-	-	26	96,0	11	41,0	1	4,0	15	56,0	13	48,0	4	15,0	10	37,0	8	30,0	2	7,0	17	63,0
San Cristóbal	4	-	-	-	-	4	100,0	1	25,0	-	-	3	75,0	3	75,0	1	25.0	-	-	3	75,0	-	-	1	25,0
Ayaccocha	23	-	-	-	-	23	100,0	10	43,0	-	-	13	57,0	15	65,0	4	17,0	4	17,0	7	30,0	1	4,0	15	65,0
Santa Ana	9	-	-	-	-	9	100,0	-	-	-	-	9	100,0	7	78,0	1	11,0	1	11,0	-	-	-	-	9	100,0
Izcuchaca	9	3	33,0	-	-	6	67,0	8	89,0	-	-	1	11,0	-	-	7	78,0	2	22,0	-	-	1	11,0	8	89,0
Yauli	7	-	-	-	-	7	100,0	1	14,0	1	14,0	5	71,0	1	14,0	1	14,0	5	71,0	-	-	-	-	7	100,0
Acoria	10	-	-	-	-	10	100,0	5	50,0	-	-	5	50,0	2	20,0	1	10,0	7	70,0	3	30,0	1	10,0	6	60,0
Total	89	4	4,0	-	-	85	96,0	36	40,0	2	2,0	51	57,0	41	46,0	19	21,0	29	33,0	21	24,0	5	6,0	63	71,0
Health center																									
Ascensión	13	1	8,0	-	-	12	92,0	4	31,0	1	8,0	8	62,0	5	38,0	3	23,0	5	38,0	4	31,0	2	15,0	7	54,0
Huachocolpa	14	-	-	-	-	14	100,0	7	50,0	-	-	7	50,0	8	57,0	1	7,0	5	36,0	4	29,0	-	-	10	71,0
San Cristóbal	4	-	-	-	-	4	100,0	1	25,0	-	-	3	75,0	3	75,0	1	25,0	-	-	3	75,0	-	-	1	25,0
Pucaccocha	8	-	-	-	-	8	100,0	2	25,0	-	-	6	75,0	5	63,0	-	-	3	38,0	4	50,0	-	-	4	50,0
Huanaspampa	3	-	-	-	-	3	100,0	1	33,0	-	-	2	67,0	2	67,0	1	33,0	-	-	3	100,0	-	-	-	-
Ayaccocha	12	-	-	-	-	12	100,0	7	58,0	-	-	5	42,0	8	67,0	3	25,0	1	8,0	-	-	1	8,0	11	92,0
Santa Ana	9	-	-	-	-	9	100,0	-	-	-	-	9	100,0	7	78,0	1	11,0	1	11,0	-	-	-	-	9	100,0
Izcuchaca	9	3	33,0	-	-	6	67,0	8	89,0	-	-	1	11,0	-	-	7	78,0	2	22,0	-	-	1	11,0	8	89,0
Yauli	7	-	-	-	-	7	100,0	1	14,0	1	14,0	5	71,0	1	14,0	1	14,0	5	71,0	-	-	-	-	7	100,0
Cochan	3	-	-	-	-	3	100,0	-	-	-	-	3	100,0	1	33,0	-	-	2	67,0	-	-	-	-	3	100,0
Acoria	3	-	-	-	-	3	100,0	2	67,0	-	-	1	33,0	1	33,0	1	33,0	1	33,0	-	-	-	-	3	100,0
Pallalla	4	-	-	-	-	4	100,0	3	75,0	-	-	1	25,0	-	-	-	-	4	100,0	3	75,0	1	25,0	-	-
Total	89	4	4,0	-	-	85	96,0	36	40,0	2	2,0	51	57,0	41	46,0	19	21,0	29	33,0	21	24,0	5	6,0	63	71,0
Age																									
One year	14	-	-	-	-	14	100,0	1	7,0	1	7,0	12	86,0	6	43,0	4	29,0	4	29,0	4	29,0	-	-	10	71,0
Two years	18	1	6,0	-	-	17	94,0	6	33,0	-	-	12	67,0	9	50,0	2	11,0	7	39,0	6	33,0	1	6,0	11	61,0
Three years	23	2	9,0	-	-	21	91,0	12	52,0	1	4,0	10	43,0	6	26,0	7	30,0	10	43,0	4	17,0	2	9,0	17	74,0
Four years	17	1	6,0	-	-	16	94,0	7	41,0	-	-	10	59,0	11	65,0	1	6,0	5	29,0	4	24,0	2	12,0	11	65,0
Five years	17	-	-	-	-	17	100,0	10	59,0	-	-	7	41,0	9	53,0	5	29,0	3	18,0	3	18,0	-	-	14	82,0
Total	89	4	4,0	-	-	85	96,0	36	40,0	2	2,0	51	57,0	41	46,0	19	21,0	29	33,0	21	24,0	5	6,0	63	71,0
Sex																									
Male	38	1	3,0	-	-	37	97,0	15	39,0	1	3,0	22	58,0	20	53,0	9	24,0	9	24,0	7	18,0	4	11,0	27	71,0
Female	51	3	6,0	-	-	48	94,0	21	41,0	1	2,0	29	57,0	21	41,0	10	20,0	20	39,0	14	27,0	1	2,0	36	71,0
Total	89	4	4,0	-	-	85	96,0	36	40,0	2	2,0	51	57,0	41	46,0	19	21,0	29	33,0	21	24,0	5	6,0	63	71,0

Legend: Oxytetracycline (OTEX), Sulfamethoxazole-trimethoprim (TSM), Chloramphenicol (C), Ceftriaxone (CRO).

Table 6. ***Antibiotic resistance of Shigella spp. isolated from children with acute gastroenteritis, by origin, age group, and sex (N=83).***

Study group	N	OTX						CRO						TSM						C					
		S		I		R		S		I		R		S		I		R		S		I		R	
		F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%	F	%
Micro-network																									
Ascensión	31	-	-	-	-	31	100,0	14	45,0	4	13,0	13	42,0	17	55,0	10	32,0	4	13,0	9	29,0	3	10,0	19	61,0
San Cristóbal	7	-	-	-	-	7	100,0	5	71,0	-	-	2	29,0	3	43,0	4	57,0	-	-	3	43,0	-	-	4	57,0
Ayaccocha	11	4	36,0	-	-	7	64,0	6	55,0	-	-	5	45,0	8	73,0	2	18,0	1	-	5	45,0	-	-	6	55,0
Santa Ana	3	-	-	-	-	3	100,0	-	-	-	-	3	100,0	3	100,0	-	-	-	-	-	-	1	33,0	2	67,0
Izcuchaca	6	-	-	-	-	6	100.0	4	67,0	-	-	2	33,0	1	17,0	4	67,0	1	-	4	67,0	-	-	2	33,0
Yauli	6	-	-	1	17,0	5	83.0	1	17,0	-	-	5	83,0	2	33,0	3	50,0	1	17,0	1	17,0	-	-	5	83,0
Acoria	19	4	21,0	-	-	15	79.0	14	74,0	-	-	5	26,0	6	32,0	8	42,0	5	26,0	9	47,0	1	5,0	9	47,0
Total	83	8	10,0	1	1,0	74	89.0	44	53.0	4	5,0	35	42,0	40	48,0	31	37,0	12	14,0	31	37,0	5	6,0	47	57,0
Health center																									
Ascensión	16	-	-	-	-	16	100,0	9	56,0	1	6,0	6	38,0	11	69,0	4	25,0	1	6,0	6	38,	2	13,0	8	50,0
Huachocolpa	15	-	-	-	-	15	100,0	5	33,0	3	20,0	7	47.0	6	40,0	6	40,0	3	20,0	3	20,0	1	7,0	11	73,0
San Cristóbal	7	-	-	-	-	7	100,0	5	71,0	-	-	2	29.0	3	43,0	4	57,0	-	-	3	43,0	-	-	4	57,0
Pucaccocha	5	-	-	-	-	5	100,0	2	40,0	-	-	3	60,0	5	100,0	-	-	-	-	2	40,0	-	-	3	60,0
Ayaccocha	6	4	67%	-	-	2	33,0	4	67,0	-	-	2	33,0	3	50,0	2	33,0	1	17,0	3	50,0	-	-	3	50,0
Santa Ana	3	-	-	-	-	3	100,0	-	-	-	-	3	100,0	3	100,0	-	-	-	-	-	-	1	33,0	2	67,0
Izcuchaca	6	-	-	-	-	6	100,0	4	67,0	-	-	2	33,0	1	17,0	4	67,0	1	17,0	4	67,0	-	-	2	33,0
Yauli	6	-	-	1	17,0	5	83.0	1	17.0	-	-	5	83.0	2	33,0	3	50,0	1	17,0	1	17,0	-	-	5	83,0
Cochan	1	-	-	-	-	1	100,0	-	-	-	-	1	100.0	-	-	1	100,0	-	-	-	-	-	-	1	100,0
Acoria	10	3	30,0	-	-	7	70,0	7	70,0	-	-	3	30,0	4	40,0	4	40,0	2	20,0	4	40,0	1	10,0	5	50,0
Pallalla	8	1	13,0	-	-	7	88,0	7	88,0	-	-	1	13.0	2	25,0	3	38,0	3	38,0	5	63,0	-	-	3	38,0
Total	83	8	10,0	1	1,0	74	89,0	44	53,0	4	5,0	35	42,0	40	48,0	31	37,0	12	14,0	31	37,0	5	6,0	47	57,0
Age																									
One year	14	2	14,0	1	7,0	11	77,	8	56,0	-	-	6	42,0	3	21,0	10	70,0	1	7,0	2	14,0	-	-	12	84,0
Two years	20	-	-	-	-	20	100,0	10	50.0	1	5,0	9	45,0	13	65,0	5	25,0	2	10,0	8	40,0	1	5,0	11	55,0
Three years	16	2	12,0	-	-	14	87,0	9	56.0	-	-	7	43,0	9	56,0	6	37,0	1	6,0	9	56,0	1	6,0	6	37,0
Four years	20	2	10,0	-	-	18	90,0	11	55.0	1	5,0	8	40,0	10	50,0	9	45,0	1	5,0	7	35,0	1	5,0	12	60,0
Five years	13	2	15,0	-	-	11	84,0	6	46.0	2	15,0	5	38,0	5	38,0	1	8,0	7	53,0	5	38,0	2	15,0	6	46,0
Total	83	8	10,0	1	1,0	74	89,0	44	53.0	4	5,0	35	42,0	40	48,0	31	37,0	12	14,0	31	37,0	5	6,0	47	57,0
Sex																									
Male	39	4	10,0	1	3,0	34	87,0	21	54,0	3	8,0	15	38,0	16	41,0	17	44,0	6	15,0	19	49,0	2	5,0	18	46,0
Female	44	4	9,0	-	-	40	91,0	23	52,0	1	2,0	20	45,0	24	55,0	14	32,0	6	14,0	12	27,0	3	7,0	29	66,0
Total	83	8	10,0	1	1,0	74	89,0	44	53,0	4	5,0	35	42,0	40	48,0	31	37,0	12	14,0	31	37,0	5	6,0	47	57,0

Legend: Oxytetracycline (OTEX), Sulfamethoxazole-trimethoprim (TSM), Chloramphenicol (C), Ceftriaxone (CRO).

DISCUSSION

Escherichia coli, Salmonella spp., Shigella spp., and other enteric bacteria are causes of acute diarrheal diseases in children under 5 years old, leading to elevated mortality rates in the poorest communities worldwide (Olaiz-Fernández et al., 2020; Palacio-Mejía et al., 2020). However, studies on the pathogenic role of these bacteria in childhood diarrhea in Peru and other Latin American countries remain limited due to the lack of detection assays in clinical laboratories in both urban and rural medical centers (De Kraker et al., 2016; Díaz-Guevara et al., 2020).

In this study, we found high prevalences of *E. coli, Salmonella spp., and Shigella spp*. in children under 5 years old with EDA. These three bacteria were frequently found across all Micro-Networks, Health Centers, and in the age groups of three to five years, predominantly in female children.

The high predominance of these three enteric bacteria in this study is attributed to contaminated water consumption (Valenza et al., 2019; Catering-Rodríguez et al., 2017), contaminated food (meat and vegetables), poor child hygiene (Díaz-Guevara et al., 2020; Catering-Rodríguez et al., 2017), and the lack of nutritional and epidemiological control and monitoring, which are critical issues in rural hospitals (Mosquito et al., 2012). These factors contribute to the massive pathogenic proliferation of enteric bacteria, as well as viruses and parasites (Vélez et al., 2015).

A study conducted in the United Kingdom and China suggested that the high prevalence and exponential transmission of *E. coli, Salmonella spp., and Shigella spp*. is due to poor diapering practices and inadequate hygienic care in hospitalized children (Bonomo et al., 2018). These practices facilitate microorganism transmission among children (Soliman et al., 2020; Zou et al., 2020), key factors in the pathogenic infection of susceptible hosts (Hertz et al., 2016). Similarly, inadequate comprehensive healthcare coverage in marginalized areas is associated with high virulence trends of enteropathogens in diarrheal cases among preschool children (Bryce et al., 2016; De Kraker et al., 2016).

The higher prevalence of first-order causative agents (*E. coli, Salmonella spp., Shigella spp*.) of EDA in female children and in the age groups of three to five years, as reported in this study, is consistent with findings from Japan, Iran, Finland, and Ghana (Olaiz-Fernández et al., 2020; Díaz-Guevara et al., 2020), which argue that anatomical features (anus and vagina) predispose females to childhood EDA due to colonization and contamination by enteropathogens (Olaiz-Fernández et al., 2020; Díaz-Guevara et al., 2020). Furthermore, these infections are associated with various factors such as protein-energy malnutrition, lack of maternal education, poor handwashing practices, absence of breastfeeding, weakened immune systems, and the socio-economic and cultural conditions of the population, which are very common in Latin American countries (Olaiz-Fernández et al., 2020; Díaz-Guevara et al., 2020).

Similarly, Gómez-Duarte (2014) reported *E. coli* as the most frequent uropathogen associated with diarrhea in children under 5 years old, while Díaz et al. (2020) found a predominance of Salmonella (54.2%) in children under 10 years old with diarrheal cases. Catering et al. (2017) isolated 4,010 *Salmonella spp*. strains from patients with EDA and Alzamora et al. (2019) identified 179 *E. coli* strains in 93 children from rural communities in Peru. Additionally, Nji et al. (2021) reported high frequencies of *E. coli* in healthy patients under 3 years of age in community settings in low- and middle-income countries.

The results of this study are similar to some previously reported findings and differ from others, such as the case of Shigella spp., which are not frequently reported despite their clinical importance in children, where they cause a spectrum of severe intestinal and extraintestinal complications that are difficult to treat once developed (Vélez et al., 2015). Therefore, further studies on this type of bacteria are warranted.

Antimicrobial resistance to different prescribed drugs in primary care is common among *E. coli*, *Salmonella spp*., and Shigella spp. carried by children with EDA (Alzamora et al., 2019; Vélez et al., 2015), with high resistance rates in developing countries that are not members of the Organization for Economic Cooperation and Development (OECD) (Bryce et al., 2016; Bonomo et al., 2018). The resistance mechanisms of these bacteria are associated with the presence of plasmids, class 1-2 integrons, beta-lactamases, and carbapenemases – CPE (Bonomo et al., 2018; Soliman et al., 2020). However, there are few reports on the antibiotic susceptibility profile of Shigella spp., with significant discrepancies, especially in Latin American countries (Olaiz-Fernández et al., 2020; Díaz-Guevara et al., 2020) due to the rise of extensive multidrug resistance in community settings (Nkansa-Gyamfi et al., 2019; Pormohammad et al., 2019).

The results showed systemic antimicrobial resistance patterns for the treatment of EDA, with *E. coli*, *Salmonella spp*., and Shigella spp. proving resistant to Oxytetracycline, Chloramphenicol, and Sulfamethoxazole-trimethoprim, making these antibiotics unsuitable therapeutic options. However, Ceftriaxone was sensitive to *E. coli* but showed contradictory results for the other bacteria studied.

The increasing multidrug resistance observed in this study could be attributed to inappropriate use and over-prescription of antibiotics in community settings (Cooke et al., 2020). Furthermore, there is a lack of awareness among healthcare personnel and the general population about the issues arising from antibiotic resistance (Olaiz-Fernández et al., 2020; Nadimpalli et al., 2018), as highlighted in the strategic objectives of the WHO's Global Action Plan on Antimicrobial Resistance (Nadimpalli et al., 2018).

Studies conducted on stool cultures in Japan by Harada et al. (2021) reported a multidrug resistance pattern in NDM-5 and OXA-48-producing E. coli, which are high-risk carbapenemase-producing clones. Similarly, Zou et al. (2020) identified 109 *E. coli* isolates resistant to carbapenem (CR-EC) and producing NDM-5 with IncF-type plasmids, leading to multidrug resistance to first-line antibiotics for EDA. Bryce et al. (2016) found high resistance rates to tetracycline (80.0–95%), ampicillin (67.2–84.9%), and trimethoprim (81.3–100%) in *E. coli* and *Salmonella spp*. strains. Díaz et al. (2020) found low sensitivity to ampicillin, nalidixic acid, oxytetracycline, chloramphenicol, and sulfamethoxazole-trimethoprim in *S. Typhi isolates*.

In Beijing, China, Yuan and Guo (2017) found multidrug resistance to ciprofloxacin, levofloxacin, and sulfamethoxazole-trimethoprim in *Salmonella Typhimurium* strains from patients with infectious diarrhea, and Al-Gallas et al. (2021) conducted comparative studies in Tunisia, finding multidrug resistance to quinolones and sulfonamides in *S. Kentucky* and *S. Typhimurium isolates*.

Studies in Peru reported high resistance rates to nalidixic acid and ciprofloxacin against *E. coli* isolates from diarrheal patients, as seen in reports by Pons et al. (2012), Montañez et al. (2015), and Mosquito et al. (2012). These findings suggest that the resistance of this uropathogen is linked to the production of BLEE, KPC, NDM-5, and OXA-48 (Bonomo et al., 2018; Soliman et al., 2020; Bryce et al., 2016; Hertz et al., 2016). A notable finding of this study was the high resistance to ceftriaxone, likely due to the presence of an BLEE-producing resistance mechanism, warranting further studies.

Other important findings included high resistance rates to oxytetracycline, chloramphenicol, ceftriaxone, and sulfamethoxazole-trimethoprim in *E. coli* and *Salmonella spp*. strains from Health Micro-Networks and Health Centers in female children under 5 years of age. For Shigella spp., oxytetracycline and chloramphenicol showed resistance, while ceftriaxone and sulfamethoxazole-trimethoprim were sensitive in some Health Micro-Networks, Health Centers, and female children aged between two and three years.

The presence of multidrug-resistant pathogenic bacteria with variations between community centers may be related to overcrowding, self-medication, poverty, socio-ecological behaviors, food security issues, highly contaminated waste effluents, inadequate surveillance systems, and the overuse of

antibiotics (Bonomo et al., 2018; Olaiz-Fernández et al., 2020; Díaz-Guevara et al., 2020), which are precursors to BLEE, KPC, NDM-5 (Soliman et al., 2020; Bryce et al., 2016).

Few studies have specifically revealed which EDA pathotypes are most frequent and their antibiotic susceptibility patterns, considering the most affected regions, climates, geographies, socio-economic statuses, and age groups in the country. Therefore, this research helps determine which populations are most at risk for these diseases and could inform the design of plans to prevent epidemiological outbreaks in the pediatric population that have not yet been reported.

CONCLUSION

The study showed high prevalences of *E. coli, Salmonella spp.,* and *Shigella spp*. in children under 5 years old with EDA, being relatively frequent in all Health Micro-Networks, Health Centers, and female patients.

Strains of *E. coli, Salmonella spp*., and *Shigella spp*. showed systemic antimicrobial resistance patterns in children under 5 years old with EDA, with ceftriaxone proving sensitive to all three bacteria, making it a viable treatment option for children with acute gastroenteritis.

CONFLICT OF INTEREST:

The authors declare no conflicts of interest.

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