

Aeromonas-Associated Diarrhea in Children under 5 Years: The GEMS Experience

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Abstract. We report the clinical findings, epidemiology, and risk factors for moderate-to-severe diarrhea (MSD) associated with *Aeromonas* species in children 0–59 months of age, from the Global Enteric Multicenter Study, conducted at three sites in south Asia and four sites in sub-Saharan Africa. Children with MSD were enrolled along with controls matched for age, gender, and neighborhood. Pooled, age-stratified conditional logistic regression models were applied to evaluate the association of *Aeromonas* infection controlling for coinfecting pathogens and socio-demographic variables. A pooled, age-stratified, multivariate logistic regression analysis was done to identify risk factors associated with *Aeromonas* positivity in MSD cases. A total of 12,110 cases and 17,291 matched controls were enrolled over a period of 48 months. *Aeromonas* was identified as a significant pathogen in 736 cases of MSD in Pakistan and Bangladesh (22.2%). *Aeromonas* remained a significant pathogen even after adjustment for the presence of other pathogens and sociodemographic factors. Odds ratio (OR) for *Aeromonas* were higher in the presence of *Shigella* (matched OR: 6.2, 95% confidence interval [CI]: 1.9–20.2). Cases of *Aeromonas* were likely to present with dysentery, particularly in the 0–11 months (OR: 1.4, 95% CI: 1.0–2.0) and 12–23 months (OR: 1.8, 95% CI: 1.3–2.5) age group. The odds of *Aeromonas* increased with increasing degree of stunting, being highest for severe stunting (OR: 10.1, 95% CI: 3.6–28.9). *Aeromonas* is a significant pathogen for MSD in Pakistan and Bangladesh. Presence of dysentery and co-occurrence with other pathogens, notably *Shigella* spp. are significant features of *Aeromonas*-associated diarrhea.

INTRODUCTION

Aeromonas species are motile, gram-negative facultative anaerobic bacilli that cause gastroenteritis. They are widespread in nature, found in soil as well as aquatic environments. They are emerging as human pathogens, and are commonly isolated from diarrheal specimens in tropical countries.¹ *Aeromonas* was first identified in human feces in 1961.² Some case–control studies have demonstrated an epidemiologic association between diarrhea in young infants and children^{3,4} while others have not.⁵ Methodological issues (e.g. short period of surveillance, controls not matched for age or season), asymptomatic shedding, and failure to consider differences in pathogenicity according to genospecies of known virulence factors may have contributed to variation in results. In various case–control studies, *Aeromonas* has been identified as a pathogen in 1–88% of children with diarrhea and in 0–45% of controls.⁶ The reported rates differ depending on the use of culture methods, species identification methods, and inclusion or exclusion of other diarrheal co-pathogens.¹ There is also a wide geographical

variation in the reported frequency of isolation and association with diarrhea.⁶

In most cases of gastroenteritis, *Aeromonas* is a co-pathogen, isolated along with other enteric pathogens.⁷ Most (90%) cases of acute *Aeromonas*-associated diarrhea (AAD) are transient and self-limiting, but dysentery and severe cholera-like diarrhea due to *Aeromonas* have been described and may require treatment with antibiotics.⁶ Despite these anecdotal descriptions, *Aeromonas* pathogenicity and virulence still remains in question.⁸

There is a need for well-designed epidemiological studies. AAD was described recently by the Global Enteric Multicenter Study (GEMS), which was designed to measure burden, microbial etiology, and nutritional effects of moderate-to-severe diarrhea (MSD) in developing countries.⁹ Attributable fractions (AFs) for enteropathogens were derived from the first 36 months of GEMS data collection. *Aeromonas* spp. were a major pathogen for diarrhea in Pakistan and Bangladesh with the highest AF among children of 24–59 months of age, where the AF for AAD in Bangladesh was 18.3 (95% confidence interval [CI]: 10.8–25.9) and for Pakistan it was 24.1 (95% CI: 16.0–32.3).¹⁰ We present the distribution of *Aeromonas* among cases and controls in children 0–59 months of age in all seven GEMS sites over 48 months and odds ratios (ORs) for *Aeromonas* MSD adjusted for other pathogens and socioeconomic factors.

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We also describe clinical and sociodemographic predictors for AAD among MSD cases.

METHODS

Primary study. The GEMS was an age-stratified, case-control study. Three age strata of 0–11, 12–23, and 24–59 months were studied. Each of seven sites in Asia and sub-Saharan Africa (Bangladesh, India, Pakistan, Mozambique, Mali, The Gambia, and Kenya) included one to 11 community Sentinel Health Centers serving censused populations. Children were defined as having diarrhea if they fit the World Health Organization definition of > 3 loose stools per day, and were included as cases if diarrhea represented a new episode (no episodes in preceding 7 days) and was moderate to severe (with any one of the following: sunken eyes, loss of skin turgor, dysentery, need for intravenous rehydration, or hospitalization). For each case of MSD, —one to three matched community controls were randomly selected from the censused population at each site. Controls were children with no episode of diarrhea in the 7 days preceding enrollment. In addition, they were selected on the basis of the following matching criteria: age + 2 months for age group 0–11 months, +4 months for age group 12–59 months, and not exceeding stratum boundaries for age group of case, same gender, residence in same catchment area, and enrollment within 14 days of that of corresponding case. Further details regarding clinical and epidemiological design of the GEMS and its Health Care Utilization and Attitudes Surveys have been described by Kotloff and others.⁹

GEMS was carried out for a total of 48 months, initially from 2009 to 2011 and for an additional 12 months in 2012.

Microbiological methods for detection of *Aeromonas* species. In GEMS, an extensive workup for a wide array of putative and established viral, bacterial, and protozoal diarrheal pathogens was undertaken on each case and control specimen at the local site laboratories, as described by Panchalingam and others.¹¹ These laboratories followed standard protocols, which were ensured by site supervisors and site visits by study personnel. Rigorous quality control and quality assurance procedures were implemented to ensure robustness of procedures.

In brief, stool specimens for detection of *Aeromonas* were collected at the field sites in Cary Blair tubes, plated on Ryan medium, and incubated aerobically at 35–37°C for 48 hours. After 48 hours, all dark green colonies with darker centers on Ryan medium were characterized further by testing for presence of oxidase, catalase, salt tolerance, and O129 susceptibility. Oxidase-positive, catalase-producing, salt-intolerant, and

O129-resistant colonies were identified as *Aeromonas* spp. Antimicrobial susceptibility tests were not performed.

Statistical analysis. Operational definitions. For the purpose of statistical analysis, *Aeromonas* positivity among cases was defined either as AAD or *Aeromonas*-only diarrhea (AOD).

AAD: All MSD cases where *Aeromonas* was isolated from stool cultures, regardless of the co-occurrence of other pathogens.

AOD: All MSD cases where *Aeromonas* was isolated as a sole pathogen.

For the parent study, a matched case-control analysis using conditional logistic regression (CLR) modeling and MSD as outcome was carried out to identify pathogens associated with MSD across the seven sites and three age strata. Adjusted ORs (AORs) were used to calculate the AF of each pathogen identified as a risk factor. These have been reported elsewhere.¹⁰ Statistical analytic methods used in the parent study have been described by Blackwelder and others.¹²

Matched case-control analysis. To evaluate the association of *Aeromonas* stool positivity with MSD, and to determine how this association is affected by other factors, we performed an age-stratified CLR analysis for Bangladesh and Pakistan. Matched AORs were calculated for *Aeromonas*. Three models were built stratified by age groups with *Aeromonas* as the main exposure variable. Model 1 was adjusted for pathogens, Model 2 was adjusted for socioeconomic variables, and Model 3 was adjusted for both pathogens and sociodemographic variables. All covariates that occurred greater than five times and had $P < 0.10$ in CLR model for individual covariate were put in the multivariable model. A backward elimination process was used to derive a parsimonious model with all variables having a P value ≤ 0.05 . All variables that co-occurred with *Aeromonas* ≥ 10 times in cases or controls were tested for interaction. All interaction terms with a P value of ≤ 0.05 were retained in the model.

Within-case logistic regression analysis. To determine clinical, microbiological, and environmental predictors of AAD, we performed a within-case logistic regression analysis for *Aeromonas* positivity among MSD cases stratified by age. We also pooled MSD cases from Bangladesh and Pakistan within their respective age strata. All variables with a P value ≤ 0.1 in the univariate analysis were used to build a multivariable model. A backward selection procedure was used to derive a parsimonious model for each age stratum, retaining only variables significant at P value ≤ 0.05 .

Ethical approval. The parent study was approved by the Ethical Review Committee of all sites and the Institutional Review Board of the University of Maryland, Baltimore, MD. Signed and written informed consents were collected from all participants. The parent or primary caretaker received a copy of the signed consent form to keep, and the original was stored in the regulatory files at the study site. Data were stored anonymously to ensure confidentiality.

RESULTS

A total of 12,110 children with MSD plus 17,291 matched controls without diarrhea were enrolled at seven sites over a period of 60 months. Figure 1 describes the distribution of *Aeromonas* spp. in stool samples of cases and controls across all sites, stratified by age group. *Aeromonas* spp.

Box 1

Lessons from the GEMS *Aeromonas* experience

- a. *Aeromonas* was significantly associated with SD in 2 of 7 GEMS sites, i.e., Pakistan and Bangladesh
- b. *Aeromonas* often presents with dysentery in children 0–59 months of age
- c. Strength of association of *Aeromonas* with MSD increases in the presence of *Shigella*
- d. Strength of association of *Aeromonas* with MSD increases with degree of stunting

GEMS = Global Enteric Multicenter study; MSD = moderate-to-severe diarrhea.

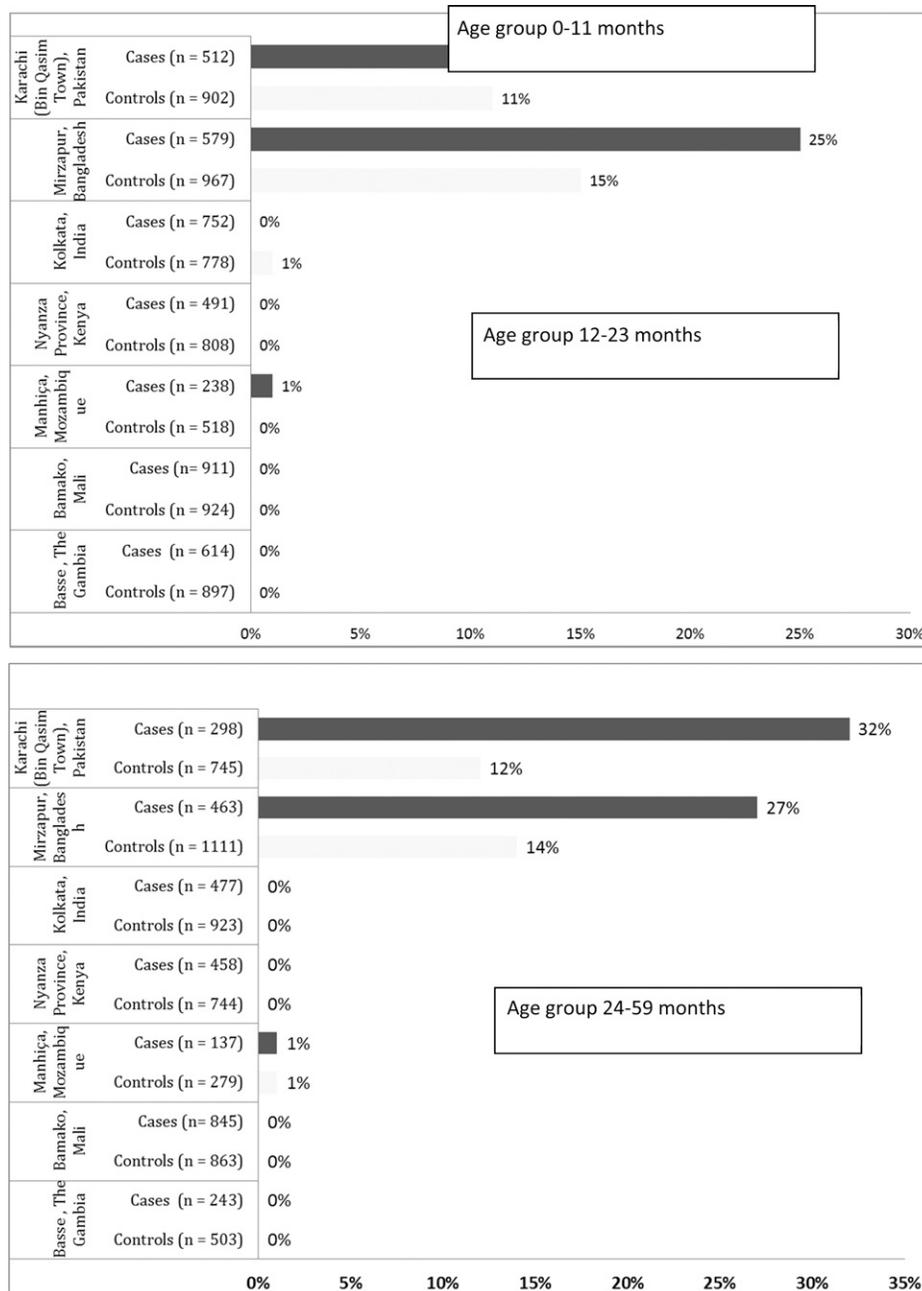


FIGURE 1. Distribution of *Aeromonas* among cases with moderate to severe diarrhea and their matched controls across seven sites and three age strata.

were recovered from stool cultures in > 1% of cases and controls from Pakistan and Bangladesh sites only.¹ Results of the matched case-control analysis from all seven GEMS sites are presented elsewhere.¹²

During the study 1,460, 1,091, and 761 MSD cases in age groups of 0–11, 12–23, and 24–59 months, respectively, were enrolled in both Pakistan and Bangladesh combined. Stool culture positivity rate for *Aeromonas* spp. among diarrheal specimens from cases was 18.7% ($N = 273$) in the 0–11 months age group, 22.5% ($N = 245$) in the 12–23 months age group, and 28.6% ($N = 218$) in the 24–59 months age group in Pakistan and Bangladesh. *Aeromonas* spp. were isolated as a sole pathogen from only 2.3% ($N = 35$) of cases aged 0–11 months,

1.8% ($N = 20$) of cases aged 12–23 months, and 4.2% ($N = 32$) of cases aged 24–59 months. Of these AOD cases, 65.7% ($N = 23$), 80% ($N = 16$), and 59.4% ($N = 19$) presented with blood in their stools from among the 0–11, 12–23, and 24–59 months age strata. Clinical features of AOD compared with MSD due to all other pathogens are provided in Supplemental Appendix, Supplemental Tables 1 and 2. Diarrheal stool specimens from cases were negative for all enteropathogens in 9.1% ($N = 133$) of 0–11 months of age group, 4.5% ($N = 50$) of 12–23 months age group, and 6.0% ($N = 46$) of 24–59 months age group.

Association of *Aeromonas* with MSD and effect of pathogens and SE factors. Table 1 presents matched ORs (mORs)

TABLE 1

Site-specific and pooled (both Pakistan and Bangladesh) mAORs for association of MSD with *Aeromonas* adjusted for pathogens and socioeconomic variables

	Bangladesh		Pakistan		Pooled	
	OR (95% CI)	P value	OR (95% CI)	P value	OR (95% CI)	P value
Model 0						
0–11	2.0 (1.5–2.6)	< 0.001	2.1 (1.5–2.9)	< 0.001	2.0 (1.7–2.5)	< 0.001
12–23	2.0 (1.5–2.7)	< 0.001	2.2 (1.6–3.0)	< 0.001	2.1 (1.7–2.6)	< 0.001
24–59	2.4 (1.8–3.2)	< 0.001	3.6 (2.5–5.0)	< 0.001	2.8 (2.3–3.5)	< 0.001
Model 1						
0–11	1.7 (1.3–2.4)	0.001	2.3 (1.5–3.4)	< 0.001	1.9 (1.5–2.5)	< 0.001
12–23	1.9 (1.2–3.0)	0.005	2.0 (1.4–2.9)	< 0.001	2.0 (1.5–2.6)	< 0.001
			<i>Aeromonas</i> × <i>Shigella</i>		<i>Aeromonas</i> × <i>Shigella</i>	
			Shigella negative, 1.8 (1.2–2.6)	0.002	Shigella negative, 1.8 (1.4–2.4)	< 0.001
			Shigella positive, 9.6 (1.8–51.3)	0.008	Shigella positive, 5.8 (1.9–18.1)	0.003
24–59	2.4 (1.5–3.9)	< 0.001	3.9 (2.6–5.9)	< 0.001	3.3 (2.4–4.5)	< 0.001
			<i>Aeromonas</i> × site		<i>Aeromonas</i> × site	
					Bangladesh, 2.3 (1.5–3.5)	< 0.001
					Pakistan, 4.8 (3.0–7.5)	< 0.001
Model 2						
0–11	1.9 (1.5–2.5)	< 0.001	2.2 (1.6–3.0)	< 0.001	2.0 (1.6–2.5)	< 0.001
			<i>Aeromonas</i> × HAZ		<i>Aeromonas</i> × age	
					≤ 6 month, 3.8 (1.7–8.2)	< 0.001
					> 6 months, 1.1 (0.6–2.3)	0.706
12–23	2.1 (1.6–2.8)	< 0.001	1.8 (1.3–2.6)	0.001	2.0 (1.6–2.6)	< 0.001
24–59	2.5 (1.8–3.4)	< 0.001	3.6 (2.3–5.4)	0.001	2.7 (2.1–3.5)	< 0.001
			<i>Aeromonas</i> × HAZ		<i>Aeromonas</i> × site	
			Well-nourished, 1.3 (0.3–4.8)	0.705	Bangladesh, 2.2 (1.3–3.5)	0.002
			Mild stunting, 1.9 (0.8–4.4)	0.128	Pakistan, 4.0 (2.5–6.6)	< 0.001
			Mod stunting, 3.6 (1.8–7.0)	< 0.001		
			Severe stunting, 11.8 (4.4–32.0)	< 0.001		
Model 3						
0–11	1.8 (1.3–2.4)	0.001	2.3 (1.6–3.5)	< 0.001	1.9 (1.4–2.5)	< 0.001
12–23	2.0 (1.2–3.1)	0.003	1.7 (1.1–2.5)	0.013	1.8 (1.3–2.5)	< 0.001
			<i>Aeromonas</i> × <i>Shigella</i>		<i>Aeromonas</i> × <i>Shigella</i>	
			Shigella negative, 1.4 (0.9–2.2)	0.097	Shigella negative, 1.6 (1.2–2.3)	0.002
			Shigella positive, 8.0 (1.5–43.8)	0.016	Shigella positive, 6.2 (1.9–20.2)	0.002
24–59	2.3 (1.4–3.9)	0.002	3.7 (2.3–6.0)	< 0.001	3.0 (2.1–4.2)	< 0.001
			<i>Aeromonas</i> × HAZ		<i>Aeromonas</i> × site	
			Well nourished, 1.0 (0.3–4.2)	0.950	Bangladesh, 2.2 (1.3–3.5)	0.002
			Mild stunting, 2.0 (0.8–5.3)	0.141	Pakistan, 4.0 (2.5–6.6)	< 0.001
			Mod stunting, 4.3 (2.1–9.0)	< 0.001		
			Severe stunting, 10.1 (3.6–28.9)	< 0.001		

CI = confidence interval; ETEC = enterotoxigenic *E. coli*; EHEC = enterohaemorrhagic *E. coli*; EAEC = entero-adherent *E. coli*; EPEC = enteropathogenic *E. coli*; HAZ = height-for-age Z score; mAORs = matched adjusted odds ratios; MSD = moderate-to-severe diarrhea; OR = odds ratio. Model 0 represents unadjusted analysis. Model 1 is adjusted for *Shigella*, *Salmonella*, *Campylobacter*, ETEC, EHEC, EAEC, EPEC, Adenovirus, *Vibrio cholera*, *Cryptosporidium*, *Giardia*, *Entamoeba histolytica*, Norovirus GI, Norovirus GII, Astrovirus, Sapovirus, and Rotavirus (cumulative list of pathogens that were adjusted across all models). Model 2 is adjusted for sociodemographic variables (wealth quintiles, water supply, treatment of drinking water, type of flooring, number of children under 5 years in household, breastfeeding, HAZ, animals in household). Model 3 is adjusted for pathogens and sociodemographic variables.

for AAD derived from four separate models. After adjusting for other enteropathogens and socioeconomic factors, *Aeromonas* remained a significant pathogen across all age strata in both sites as well as in the pooled analysis. In the model adjusted for both other pathogens and sociodemographic variables (Model 3), *Aeromonas* had an interaction with *Shigella* species driven by the Pakistan site in age group 2. The mORs for *Aeromonas* were higher in the presence of *Shigella* (mOR: 1.4, 95% CI: 0.9–2.2 versus mOR: 8.0, 95% CI: 1.5–43.8) in Pakistan. This interaction was also seen when data from Bangladesh and Pakistan were pooled. In age group 3, there was an interaction with nutritional status at Pakistan site, mOR for *Aeromonas* increased with increasing degree of stunting (Figure 2).

In age group 3, mOR for *Aeromonas* was higher in Pakistan than in Bangladesh (mOR: 3.7, 95% CI: 2.3–6.0 versus mOR: 2.3, 95% CI: 1.4–3.9). This also manifested as an interaction with site in the pooled analysis.

Clinical, microbiological, and environmental predictors of AAD. Table 2 presents results for the multivariate analysis

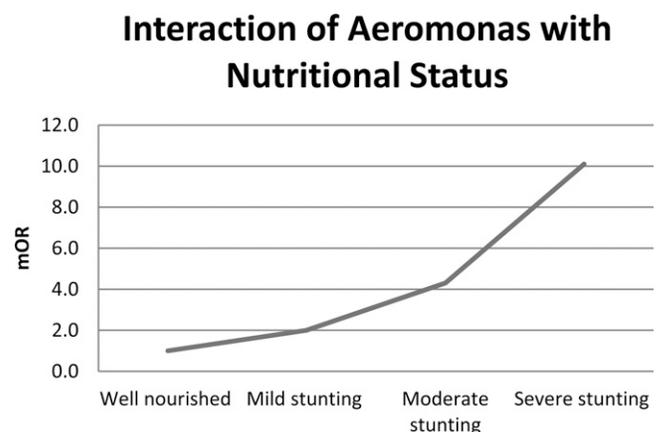


FIGURE 2. Interaction of moderate-to-severe diarrhea due to *Aeromonas* with nutritional status (height-for-age Z score) in children aged 24–59 months in Pakistan.

TABLE 2
Multivariate model of predictors of *Aeromonas* positivity among MSD cases in Bangladesh and Pakistan

	0–11 months cases (N = 1,460)				12–23 months cases (N = 1,091)				24–59 months cases (N = 761)			
	OR	95% CI		P value	OR	95% CI		P value	OR	95% CI		P value
		Lower	Upper			Lower	Upper			Lower	Upper	
Model 1												
<i>Shigella</i> species	1.6	1.1	2.3	0.023	1.5	1.1	2.0	0.010	–	–	–	–
NTS	2.87	1.62	5.09	< 0.001	–	–	–	–	–	–	–	–
Model 2												
Breastfed	0.4	0.3	0.6	< 0.001	0.6	0.4	0.9	0.008	–	–	–	–
Dysentery	1.5	1.1	2.1	0.022	2.1	1.5	2.9	< 0.001	–	–	–	–
Abdominal pain	1.5	1.1	2.1	0.019	–	–	–	–	–	–	–	–
Rehydrated with homemade fluid	2.8	1.2	6.4	0.016	–	–	–	–	–	–	–	–
Model 3												
NTS	2.1	1.1	4.0	0.026	–	–	–	–	–	–	–	–
Breastfed	0.4	0.3	0.6	< 0.001	0.6	0.4	0.9	0.024	–	–	–	–
Dysentery	1.4	1.0	2.0	0.04	1.8	1.3	2.5	0.001	–	–	–	–
Abdominal pain	1.5	1.1	2.1	0.026	–	–	–	–	–	–	–	–
Rehydrated with homemade fluid	2.6	1.1	6.0	0.025	–	–	–	–	–	–	–	–

CI = confidence interval; NTS = nontyphoidal *Salmonella*; OR = odds ratio.

for predictors of AAD, pooled for Bangladesh and Pakistan. Site-specific analysis is presented as a Supplemental Appendix, Supplemental Tables 1 and 2. In the 0–11 months age group, AAD cases were more likely to be not breastfed (OR: 0.4, 95% CI: 0.3–0.6), present with dysentery (OR: 1.4, 95% CI: 1.0–2.0) and abdominal pain (OR: 1.5, 95% CI: 1.1–2.1), more likely to have received home-based fluid therapy (OR: 2.6, 95% CI: 1.1–6.0), and more likely to co-occur with nontyphoidal *Salmonella* (NTS) (OR: 2.1, 95% CI: 1.1–4.0). In 12–23 months age group, AAD cases were again more likely to be not breastfed (OR: 0.6, 95% CI: 0.4–0.9) and more likely to present with dysentery (OR: 1.8, 95% CI: 1.3–2.5). There were no significant predictors identified in the third age group.

Speciation of *Aeromonas* was performed by the Sanger Institute, and 131 *Aeromonas* isolates were sequenced. Preliminary data show that they belong to the following species: *Aeromonas caviae* (42 cases and 42 controls), *Aeromonas dhakensis* (7 cases and 3 controls), *Aeromonas veronii* (11 cases and 17 controls), *Aeromonas enteropelogenes* (4 cases and 2 controls), and *Aeromonas sanarelli* (1 case and 0 control). The sequence of one isolate did not match any of the 141 references present in the databases.

DISCUSSION

Aeromonas was significantly associated with MSD among children less than 5 years of age at two Asian sites: Karachi, Pakistan, and Mirzapur, Bangladesh. *Aeromonas* was significantly associated with MSD in all age groups and after controlling for the presence of co-pathogens and for socio-demographic variables that might increase the risk of MSD. Our findings further illustrate a remarkable geographic variation in the importance of *Aeromonas* as a diarrheal pathogen. While *Aeromonas* was identified in < 1% of cases and controls in all age strata from the African sites and Kolkata, India, it was identified in 18.7% of infants, 22.5% of toddlers 12–23 months of age, and 28.6% of children 24–59 months of age. The association was seen at the two sites individually and when the data were pooled, all with a trend toward increasing frequency with age.

Past studies evaluating whether *Aeromonas* is significantly associated with childhood diarrhea have yielded conflicting results. Some case–control studies have demonstrated an epidemiologic association with diarrhea in infants and children³ while others have not.^{5,13} Factors that may have contributed to study-to-study variation include methodological issues (e.g. short period of surveillance, failure to account for differences in cases and controls in factors such as age or season), carriage rates in asymptomatic children that were similar to diarrhea patients in some settings, geographic variability in disease incidence, and failure to account for differences in pathogenicity according to genospecies or known virulence factors. In addition, bias may be introduced when the impact of coinfection with other pathogens is not considered. Finally, there may be host factors that influence the pathogenicity of *Aeromonas* in various settings, such as genetic predilection, the prevalence of underlying stunting, a risk factor in our study, or high environmental exposure due to predisposing factors such as contamination of drinking water. GEMS overcame many of these issues by using standardized methods in a 36-month study conducted at seven sites in Africa and Asia and performing analyses that controlled for the presence of co-pathogens and evaluated associations in episodes from which *Aeromonas* was the only pathogen identified. The absence of these confounding factors strengthens our epidemiologic observation that *Aeromonas* is significantly associated with watery diarrhea and dysentery among children under 5 years of age in Bangladesh and Pakistan.

Of the several risk factors for *Aeromonas* evaluated in GEMS, we were able to identify lack of breastfeeding as a significant risk factor in pooled analysis from Pakistan and Bangladesh. Breast milk contains factors such as secretory IgA and oligosaccharides that prevent both pathogen-specific (e.g., *Shigella* and rotavirus) and nonspecific diarrheal illness. Moreover, reliance of breastfeeding may prevent exposure to contaminated food and water. A lack of breastfeeding necessitates feeding with either animal-source milk, which is diluted with water before being fed to babies, or other food prepared with water.

An interesting feature of AAD is the occurrence of co-pathogens. In the present study, NTS and *Shigella* spp.

showed a significant association with AAD particularly in the younger age groups. In some studies *Aeromonas* is commonly part of a polymicrobial infection, which raises the question of whether it is behaving as a nonpathogenic “co-traveler” perhaps acquired from the same source as the true pathogen, or is interacting with other agents to cause disease. Pathogens commonly reported in literature to coexist with *Aeromonas* are rotavirus, *Vibrio* spp., *Shigella*, *Campylobacter*, and *Escherichia coli* O157:H7.^{4,13–15}

Overall, *Aeromonas* comprised 22.7% of episodes of watery diarrhea and 26.3% of episodes of dysentery at these two sites. The proportion of cases manifesting as bloody diarrhea increased with age, from 42.4% of infants to ~754.6% of those 1 year or older. However, *Aeromonas* was associated with dysentery only in the lower two age strata. This association between *Aeromonas* spp. and dysentery has been observed by others.^{16,17} Lending biological plausibility to the relationship between *Aeromonas* and dysentery is the observation that *Aeromonas* isolates from two patients with dysentery were invasive and able to replicate intracellularly in HEP-2 cell assays.¹⁸ Moreover, a cytotoxic enterotoxin Act from a human *Aeromonas hydrophila* isolate causes epithelial destruction in mouse ligated intestinal loops¹⁹ and elicits inflammatory responses, features that might produce dysentery in humans.

There are several limitations of this study. It is possible that there were coinfecting organisms such as *Shigella* that were not cultivated but were responsible for some proportion of the diarrheal symptoms observed. Further investigations using molecular diagnostics in children with MSD and their controls may shed additional light on this issue. The parent study was designed to detect pathogens for MSD and not powered for risk factors for individual pathogens. Second, although we know from reviewing the literature that *A. hydrophila*, *Aeromonas sobria*, and *A. caviae* are the species most frequently implicated in diseases of humans, we did not perform speciation of *Aeromonas*. Although this is unlikely to affect the results of the study, speciation may help to differentiate environmental from pathogenic strains.

CONCLUSIONS

Aeromonas is significantly associated with MSD, causing both watery diarrhea and dysentery among children under 5 years of age in Pakistan and Bangladesh but not in other geographic areas in Asia and sub-Saharan Africa, demonstrating its considerable geographic variability. Risk factors for AAD are similar to those of other bacterial pathogens. Presence of dysentery and co-occurrence with *Shigella* are significant features of AAD.

In the face of continued resource constraints and poor policy making, low-cost, “enabling” measures such as organized health education at community level to promote breastfeeding and standardized home-based water purification can be effective in AAD prevention. Furthermore, large community-based, pathogen-specific studies are important to understand the epidemiology and distribution of AAD to enable development of effective programs for disease prevention.

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REFERENCES

1. Ghenghesh KS, Ahmed SF, El-Khalek RA, Al-Gendy A, Klena J, 2008. *Aeromonas*-associated infections in developing countries. *J Infect Dev Ctries* 2: 81–98.
2. Lautrop H, 1961. *Aeromonas hydrophila* isolated from human feces and its possible pathological significance. *Acta Path Microbiol Scand* 51 (Suppl 144): 299–301.
3. Burke V, Gracey M, Robinson J, Peck D, Beaman J, Bundell C, 1983. The microbiology of childhood gastroenteritis: *Aeromonas* species and other infective agents. *J Infect Dis* 148: 68–74.
4. Pazzaglia G, Sack RB, Salazar E, Yi A, Chea E, Leon-Barua R, Guerrero CE, Palomino J, 1991. High frequency of coinfecting

- enteropathogens in *Aeromonas*-associated diarrhea of hospitalized Peruvian infants. *J Clin Microbiol* 29: 1151–1156.
5. Kotloff KL, Wasserman SS, Steciak JY, Tall BD, Losonsky GA, Nair P, Morris JG Jr, Levine MM, 1988. Acute diarrhea in Baltimore children attending an outpatient clinic. *Pediatr Infect Dis J* 7: 753–759.
 6. Von Graevenitz A, 2007. The role of *Aeromonas* in diarrhea: a review. *Infection* 35: 59–64.
 7. Albert MJ, Ansaruzzaman M, Talukder KA, Chopra AK, Kuhn I, Rahman M, Faruque ASG, Islam MS, Sack RB, Mollby R, 2000. Prevalence of enterotoxin genes in *Aeromonas* spp. isolated from children with diarrhea, healthy controls, and the environment. *J Clin Microbiol* 38: 3785–3790.
 8. Janda JM, Abbott SL, 2010. The genus *Aeromonas*: taxonomy, pathogenicity, and infection. *Clin Microbiol Rev* 23: 35–73.
 9. Kotloff KL, Blackwelder WC, Nasrin D, Nataro JP, Farag TH, van Eijk A, Adegbola RA, Alonso PL, Breiman RF, Faruque ASG, 2012. The Global Enteric Multicenter Study (GEMS) of diarrheal disease in infants and young children in developing countries: epidemiologic and clinical methods of the case-control study. *Clin Infect Dis* 55: S232–S245.
 10. Kotloff KL, Nataro JP, Blackwelder WC, Nasrin D, Farag TH, Panchalingam S, Wu Y, Sow SO, Sur D, Breiman RF, 2013. Burden and aetiology of diarrhoeal disease in infants and young children in developing countries (the Global Enteric Multicenter Study, GEMS): a prospective, case-control study. *Lancet* 382: 209–222.
 11. Panchalingam S, Antonio M, Hossain A, Mandomando I, Ochieng B, Oundo J, Ramamurthy T, Tamboura B, Zaidi AKM, Petri W, 2012. Diagnostic microbiologic methods in the GEMS-1 case/control study. *Clin Infect Dis* 55: S294–S302.
 12. Blackwelder WC, Biswas K, Wu Y, Kotloff KL, Farag TH, Nasrin D, Graubard BI, Sommerfelt H, Levine MM, 2012. Statistical methods in the Global Enteric Multicenter Study (GEMS). *Clin Infect Dis* 55: S246–S253.
 13. Figura N, Marri L, Verdiani S, Ceccherini C, Barberi A, 1986. Prevalence, species differentiation, and toxigenicity of *Aeromonas* strains in cases of childhood gastroenteritis and in controls. *J Clin Microbiol* 23: 595–599.
 14. Dixit S, Bhandari GP, Karmacharya DB, Shrestha S, Manandhar S, Maskey MK, 2011. Molecular screening of major bacterial enteropathogens in human stool samples from diarrhoeal outbreak sites. *J Nepal Health Res Counc* 9: 181–185.
 15. Sinha S, Shimada T, Ramamurthy T, Bhattacharya SK, Yamasaki S, Takeda Y, Nair GB, 2004. Prevalence, serotype distribution, antibiotic susceptibility and genetic profiles of mesophilic *Aeromonas* species isolated from hospitalized diarrhoeal cases in Kolkata, India. *J Med Microbiol* 53: 527–534.
 16. Soltan Dallal MM, Moezardalan K, 2004. *Aeromonas* spp associated with children's diarrhoea in Tehran: a case-control study. *Ann Trop Paediatr* 24: 45–51.
 17. Klontz EH, Faruque AS, Das SK, Malek MA, Islam Z, Luby SP, Klontz KC, 2012. Clinical and epidemiologic features of diarrheal disease due to *Aeromonas hydrophila* and *Plesiomonas shigelloides* infections compared with Those due to *Vibrio cholerae* Non-O1 and *Vibrio parahaemolyticus* in Bangladesh. *ISRN Microbiol* 2012: 654819.
 18. Lawson MA, Burke V, Chang BJ, 1985. Invasion of HEP-2 cells by fecal isolates of *Aeromonas hydrophila*. *Infection and Immunity* 47: 680–683.
 19. Xu X-J, Ferguson MR, Popov VL, Houston CW, Peterson JW, Chopra AK, 1998. Role of a cytotoxic enterotoxin in *Aeromonas*-mediated infections: development of transposon and isogenic mutants. *Infection and Immunity* 66: 3501–3509.