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Fecal Contamination of Drinking Water Was Associated with Diarrheal Pathogen Carriage among Children Younger than 5 Years in Three Peruvian Rural Communities

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Abstract. Drinking water contamination is a frequent problem in developing countries and could be associated with bacterial pathogen carriage in feces. We evaluated the association between the risk of drinking water and bacterial carrier status in children younger than 5 years in a cross-sectional study conducted in 199 households from three Peruvian rural communities. Fecal samples from children were screened for pathogenic *Aeromonas*, *Campylobacter*, and *Vibrio* species, as well as for Enterobacteriaceae, including pathogenic *Escherichia coli*. The drinking water risk was determined using *E. coli* as an indicator of contamination. Nineteen (9.5%) children were colonized with pathogens and classified as carriers, all without diarrhea symptoms. Of 199 drinking water samples, 38 (19.1%) were classified as very high risk because of high fecal contamination (> 100 *E. coli*/100 mL). Shared-use water sources, daily washing of containers, and washing using only water were associated with higher prevalence of bacterial carriage, whereas there was no association between households reporting boiling and chlorination of water and carrier status. The prevalence of carriage in children exposed to very high-risk water was 2.82 (95% CI: 1.21–6.59) times the prevalence of those who consumed less contaminated water, adjusted by the water source and daily washing. Our results suggest that household drinking water plays an important role in the generation of carriers with diarrheal pathogens. Our findings also highlight the importance of interventions to ensure the safety of drinking water. Further studies are needed to validate the observed association and determine its significance with respect to diarrhea in the community.

INTRODUCTION

Fecal contamination of drinking water is a frequent problem in developing countries.[1–7] Contaminated water represents a health threat as it can be a vehicle for pathogen transmission.[2,8–10] The association between contaminated drinking water and cholera is clear[11,12] but does not apply equally to all diarrheal pathogens.[12]

The risk of diarrheal disease in children younger than 5 years can be reduced through interventions that promote access to clean water and sanitary facilities, hygiene practices, and water treatment.[6,7,12,13] In rural areas of Peru, despite local efforts and interventions to promote clean and safe water, approximately 95% of children younger than 5 years are exposed to drinking water contaminated with fecal coliforms.[14–17]

Enterobacteriaceae, as well as *Campylobacter* and *Aeromonas* species are frequent causes of diarrhea that affect children younger than 5 years.[18–20] The presence of pathogenic bacteria in children without diarrheal symptoms has been described previously, suggesting that a carrier state may exist among children exposed early to these pathogens.[15,21] However, the literature about risk factors that may lead to this carrier state is limited, particularly in pediatric populations.

Several studies have shown that unsafe drinking water is associated with gastrointestinal infections.[6,7,22] Researchers have also suggested that consumption of contaminated water may be associated with a carrier state of pathogenic bacteria.[23,24] It is possible that children exposed to frequent consumption of contaminated drinking water will develop an immunity and tolerance to frequently consumed bacterial pathogens that may lead to asymptomatic carriage. Therefore, we aimed to evaluate the association between contaminated household drinking water and the carriage of pathogenic bacteria in children younger than 5 years.

MATERIALS AND METHODS

Study population and participants. The study was conducted in Independencia (13°41′34.5″S 76°01′32.9″W), Bernales (13°44′42.3″S 75°57′54.2″W), and Huancano (13°36′05.1″S 75°37′11.0″W), all located in a rural area of the Pisco Province of the Peruvian southern coast. In August 2007, an earthquake occurred in Pisco affecting 70% of its homes and the water distribution system and sewage systems of the communities included in this study.[25] Three years later, these three communities still had intermittent and limited access to chlorinated water through domestic pipes for some hours during the day.[26]

Study design and data collection. A cross-sectional study was conducted in 199 households during August 2010. Researchers from the United States Naval Medical Research Unit No. 6 (NAMRU-6) in collaboration with Peace Corps Volunteers, public health authorities, community leaders, and members of the "Vaso de Leche" program of Independencia, Bernales, and Huancano identified 450 households distributed among the three communities where at least one child younger than 5 years lived (referred to as child henceforth). A total of 199 (44.2%) households were randomly selected in a sampling with proportional probability to the population size of each community. One child was enrolled in each household, selected randomly from among the children in each household with more than one eligible child. Assessments in each house involved 1) a survey of sociodemographic characteristics, hygiene practices, and water treatment practices; 2) a stool sample from the enrolled child; and 3) a sample of the household drinking water.

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Survey. The survey questionnaire was designed using questions from the Peruvian Demographic Health Survey[27] and was evaluated in a pilot study and validated in a neighboring community with similar characteristics to the communities included in this study. The survey consisted of 45 questions which took approximately 30 minutes to complete. Field workers were trained and evaluated in the survey administration. After verbal informed consent was obtained, the survey was administered to either the child's mother or to a caregiver if the mother was absent.

Stool samples. After the survey administration, field workers provided instructions to the mother/caregiver for collecting stool sample from the child. Briefly, the child defecated into a clean and sterile container provided by the field workers or in a diaper if collection in the container was not possible. Then, the mother/caregiver stored the feces in a sterile screw-top container for delivery to the field worker. The stool sample was retrieved by the field workers within 24 hours from sample collection. Once the sample was received, the field worker selected a representative part of the sample (~2.0 g) and inoculated it into Cary-Blair transport medium. Stool samples were then transported daily at 4°C from each community to the NAMRU-6 laboratory in Lima, Peru.

Drinking water samples. Field workers were trained and evaluated on the drinking water sample collection procedures. On the day of the survey, the field worker asked the mother/caregiver to identify the principal drinking water container used for the child's water consumption. If there were multiple containers, then one would be randomly selected. The field worker would then collect 100 mL of water from the selected container in a sterile 100-mL bottle to be transported that day at 4°C to the laboratory at NAMRU-6.

Laboratory methods. *Stool samples.* Stool samples were tested for the presence of pathogenic Enterobacteriaceae and species of *Aeromonas*, *Campylobacter*, and *Vibrio* using MacConkey, Hektoen, XLD, Campy, and TCBS plates. Culture-based methods are still considered as the gold standard for detection of pathogenic bacteria. In addition, real-time PCR assays (100.0% specific and 99.0% sensitive) were performed to detect six classes of pathogenic *Escherichia coli*.[28]

Drinking water samples. The presence of *E. coli* was tested in drinking water samples and quantified using the most probable number in 100 mL using the Quanti-Tray 200, Colilert kit (Idexx Laboratories, Westbrook, ME), following the manufacturer's instructions.

Ethical approvals. The study was approved by NAMRU-6's IRB (NMRCD.2009.0006). The mothers and caregivers agreed to participate through informed consent and also approved the child's enrollment. This study was also approved by the Universidad Peruana Cayetano Heredia as the Master's thesis of the lead author (SIDISI: 66909).

Measures and analysis. *Outcome variable.* The carriage of pathogenic bacteria was defined dichotomously (yes/no). Children with at least one type of pathogenic bacteria detected in the stool sample were classified as carriers.

Exposure of interest. Drinking water samples with > 100 *E. coli*/100 mL were classified as "very high risk" following the WHO's health risk categories.[29] Drinking water with no detectable *E. coli* or with 1–100 *E. coli*/100 mL was classified as "not very high risk."[29,30]

Main covariate. The other covariates were grouped into 1) child demographics (gender and age) and malnutrition (global, chronic, and acute malnutrition), 2) socioeconomic status and water source, 3) hygiene practices (daily washing of the drinking water storage container and cleaning supplies), and 4) water treatment methods (boiling and chlorination). Global, chronic, and acute malnutrition were defined dichotomously if a child's indices of weight–age, height–age, and weight–height were 2 SDs below the reference population's median, respectively.[31] A socioeconomic status index was estimated using a principal component analysis of assets and household characteristics.[32,33] The water source type was dichotomized post hoc as "piped," if the house had access to water through a domestic pipe, or as a "shared-use" water source if the house only had access to a well or standpipe in the community. The frequency of washing the drinking water storage container was originally collected as an ordinal variable (number of times cleaned per week) but was analyzed as a dichotomous "daily wash" (yes/no) variable because of the high variability of its values.

Statistical analysis. The associations between carrier status and categorical variables were explored using Fisher's exact test. The ages of carriers and noncarriers were compared using the Mann–Whitney U test. Crude prevalence ratio (PR) and adjusted PR (aPR) of carriage and their 95% CIs were estimated using generalized linear models with a Poisson distribution, logarithmic function, and robust estimations of standard errors. Binomial distribution was not used because of the lack of convergence in multiple regression models.[34–36] The variance inflation factor was evaluated to assess multicollinearity among covariates. The consistency of PR estimates for high-risk drinking water and carrier status was evaluated across all fitted multiple regression models. Data analysis was performed using Stata v14 (StataCorp, College Station, TX) considering a statistical significance of $P < 0.05$.

RESULTS

Characteristics of the study population. A total of 199 children from Bernales (53.7%), Huancano (16.1%), and Independencia (30.2%) were enrolled. The male:female ratio was 0.9, and the median age was 2.4 years (Table 1). Pathogenic bacteria were detected in the stool of 19 children (9.5%), who were classified as carriers. The carriers were detected in all three communities; seven from Bernales, one from Huancano, and 11 from Independencia. One child was carrying two pathogenic bacteria simultaneously and 18 were single carriers, resulting in a total of 20 bacterial isolates. *Campylobacter jejuni* was the most frequently isolated bacteria (7/20, 35.0%), followed by *Aeromonas caviae* (6/20, 30.0%), *Campylobacter coli* (3/20, 15.0%), *Aeromonas hydrophila* (2/20, 10.0%), *Aeromonas veroni* (1/20, 5.0%), and *Shigella flexneri* (1/20, 5.0%). No pathogenic *E. coli* were detected.

The mothers/caregivers of the carrier children reported that their child did not have diarrheal disease symptoms in the 3 days before enrollment. No children younger than 1.3 years were carriers of pathogenic bacteria (Table 1). We did not observe associations between gender, age, malnutrition, or socioeconomic status with carriage (Table 1).

Water and hygiene practices. Of the 199 households, 177 (88.9%) reported having a water connection inside the house used as the primary water source, whereas 22 (11.1%) reported shared-use water sources because of lack of in-house plumbing. Whether domestic pipes or shared water sources,

TABLE 1
Characteristics difference between noncarriers and carriers of enteric bacterial pathogens of enrolled children younger than 5 years

		Noncarrier (*n* = 180)	Carrier (*n* = 19)	
	% (*n*/*N*)	*n* (%)	*n* (%)	*P*-value
Gender				0.470
Female	52.8 (105/199)	93 (88.6)	12 (11.4)	
Male	47.2 (94/199)	87 (92.5)	7 (7.5)	
Age (years)	2.4 (0.1–4.9)*	2.3 (0.1–4.9)*	2.9 (1.3–4.5)*	0.172†
< 1	13.1 (26/199)	26 (100.0)	0 (0.0)	0.134
1 to < 2	26.1 (52/199)	45 (86.5)	7 (13.5)	
≥ 2 to < 5	60.8 (121/199)	109 (90.1)	12 (9.9)	
Global malnutrition				0.397
No	95.5 (190/199)	171 (90.0)	19 (10.0)	
Yes	4.5 (9/199)	9 (100.0)	0 (0.0)	
Chronic malnutrition				0.321
No	83.9 (167/199)	149 (89.2)	18 (10.8)	
Yes	16.1 (32/199)	31 (96.9)	1 (3.1)	
Acute malnutrition				1.000
No	98.5 (196/199)	177 (90.3)	19 (9.7)	
Yes	1.5 (3/199)	3 (100.0)	0 (0.0)	
Socioeconomic status				0.051
Lower	34.6 (69/199)	62 (89.9)	7 (10.1)	
Half	32.7 (65/199)	55 (84.6)	10 (15.4)	
Upper	32.7 (65/199)	63 (96.9)	2 (3.1)	
Water source‡				< 0.001
Piped at home	88.9 (177/199)	167 (94.9)	9 (5.1)	
Shared	11.1 (22/199)	12 (54.6)	10 (45.4)	
Daily washing of water storage container				< 0.001
No	67.3 (134/199)	130 (97.0)	4 (3.0)	
Yes	32.7 (65/199)	50 (76.9)	15 (23.1)	
Cleaning supplies for washing‡				0.043
Water	28.3 (56/199)	46 (82.1)	10 (17.9)	
Water + dishwashing	38.4 (76/199)	70 (92.1)	6 (7.9)	
Water + bleach	33.3 (66/199)	63 (95.5)	3 (4.5)	
Boiling drinking water				0.480
No	13.6 (27/199)	26 (96.3)	1 (3.7)	
Yes	86.4 (172/199)	154 (89.5)	18 (10.5)	
Chlorinating drinking water				0.811
No	53.8 (107/199)	96 (89.7)	11 (10.3)	
Yes	46.2 (92/199)	84 (91.3)	8 (8.7)	
Very high-risk water				< 0.001
No	80.9 (161/199)	152 (94.4)	9 (5.6)	
Yes	19.1 (38/199)	28 (73.7)	10 (26.3)	

* p50 (minimum–maximum).
† Mann–Whitney U test.
‡ One missing value.

all families reported storing water mainly for drinking and food preparation in plastic containers. Approximately one-third (32.7%) of containers used to store drinking water were washed daily. Containers were most frequently washed with a combination of water and dishwashing soap (38.4%). Most households also reported some form of treatment for drinking water, either by boiling (86.4%) and/or chlorination (46.2%).

We observed a significantly higher carriage frequency in those households that used shared water sources, reporting daily washing of containers, and reporting washing containers with only water (Table 1). Reports of water boiling and chlorination were not associated with carrier status (Tables 1 and 2).

In the multiple regression model, the water source type and daily container washing were independently associated with carrier status (Table 2). The carriage PR associated with using shared water sources compared with in-house pipes was 6.06 (95% CI: 2.71–13.58), after adjusting for daily washing. Also, the carriage prevalence in households reporting daily container washing was higher than for non-daily washing (PR: 5.48, 95% CI: 1.85–16.21), after adjustment by the water source. We did not observe associations between carriage and child characteristics or water treatment methods in multiple regression models adjusting for water source and daily washing.

A total of 38 (19.1%) drinking water samples were classified as very high risk based on the level of *E. coli* contamination (Table 1). No statistical difference was observed in the *E. coli* load by water source type ($P = 0.233$, Figure 1). The carriage prevalence was higher in children exposed to very high-risk drinking water (PR = 4.71, 95% CI: 2.05–10.80, Table 2). Adjusting for the water source and daily container washing, the carriage prevalence was still higher in children exposed to the very high-risk drinking water (aPR: 2.82, 95% CI: 1.21–6.59, Table 2). The association between drinking water risk and carrier status was stable in consistency analyses despite multiple statistical adjustments (Table 3).

DISCUSSION

We observed a strong association between very high-risk drinking water and carrier status with at least 2.6-fold higher prevalence of pathogenic bacteria carriage. The increase in

TABLE 2

Prevalence ratios comparing children characteristics and water and hygiene practices between carriers and noncarriers of enteric bacterial pathogens

	Unadjusted PR			aPR 1*			aPR 2†		
	PR	95% CI	*P*-value	aPR	95% CI	*P*-value	aPR	95% CI	*P*-value
Gender									
Female	Ref.			Ref.			Ref.		
Male	0.65	0.27–1.59	0.347	0.62	0.27–1.38	0.238	0.6	0.28–1.29	0.188
Chronic malnutrition									
No	Ref.			Ref.			Ref.		
Yes	0.29	0.04–2.11	0.221	0.27	0.04–1.62	0.152	0.33	0.07–1.63	0.174
Socioeconomic status									
Lower	0.66	0.27–1.63	0.368	1.07	0.47–2.44	0.875	1.08	0.51–2.25	0.846
Half	Ref.			Ref.			Ref.		
Upper	0.2	0.05–0.88	< 0.001	0.25	0.06–1.04	0.057	0.3	0.08–1.18	0.085
Water source									
Piped at home	Ref.			–			–		
Shared	8.89	4.05–19.51	< 0.001	–	–		–	–	–
Daily washing of water storage container									
No	Ref.			Ref.			–		
Yes	7.73	2.66–22.43	< 0.001	5.46	1.85–16.21	0.002	–	–	–
Cleaning supplies for washing									
Water	Ref.			Ref.			Ref.		
Water + dishwashing	0.44	0.17–1.15	0.094	0.43	0.13–1.48	0.181	0.51	0.24–1.13	0.099
Water + bleach	0.03	0.07–0.88	0.031	0.58	0.24–1.39	0.218	0.58	0.15–2.16	0.461
Boiling drinking water									
No	Ref.			Ref.			Ref.		
Yes	2.82	0.39–20.41	0.303	2.35	0.42–13.05	0.328	1.8	0.23–13.85	0.572
Chlorinating drinking water									
No	Ref.			Ref.			Ref.		
Yes	0.85	0.35–2.02	0.706	1.12	0.51–2.49	0.772	1.22	0.48–2.55	0.605
Very high-risk water									
No	Ref.			Ref.			Ref.		
Yes	4.71	2.05–10.80	< 0.001	3.46	1.52–7.87	0.003	2.82	1.21–6.59	0.017

aPR = adjusted prevalence ratio; PR = prevalence ratio.
* Adjusted with water source.
† Adjusted for water source and daily washing.

carrier status was stable despite multiple statistical approaches looking for confounding factors and even reached a 4-fold increase in some scenarios. This association was previously described by Coleman et al.[23] and suggested that exposure to very highly contaminated water may lead to the generation of bacterial pathogen carriers.

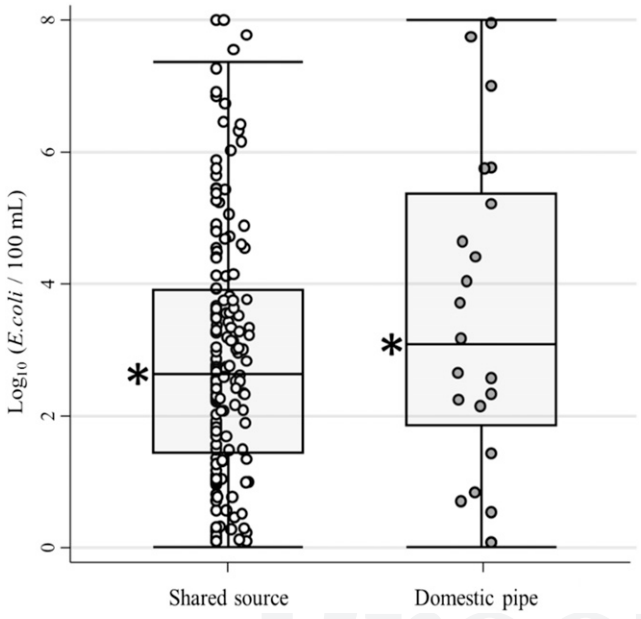

FIGURE 1. Load of *Escherichia coli* in drinking water by the type of water source.

Nearly 10% of children sampled were positive for pathogenic bacteria, similar to results from a rural Peruvian community[15] and other previous reports.[19,20] It is very probable that the cases could have developed the disease in subsequent days, or the pathogen have been eliminated without causing disease, or that they served as reservoirs of pathogenic bacteria increasing the risk of contaminating their surroundings. This is especially significant in crowded settings such as day-care facilities and schools as caregivers are less attentive to good hygiene practices.[37] As previously described,[38] quantitative approaches to distinguish high- and low-level infections, serological evidence to estimate force of infection, and longitudinal studies to confirm the carrier status or symptomatic infections are needed to validate the association described here and also to better understand the burden of asymptomatic carriers in high-level infections.

The relatively high prevalence of positive cases for pathogenic bacteria with no symptoms of disease, its presence in multiple communities, and the fact that it is associated with multiple pathogens is associated with highly contaminated drinking water present in almost 20% of homes suggest that exposure to pathogenic bacteria is not a single, isolated event but rather a somewhat continuous situation at the community level. Interestingly, all children carrying pathogenic bacteria in this population were older than 1 year. The mechanism and frequency of such tolerance to disease as well as to see if age and age-related characteristics are associated to carrier status and symptoms merit further research.

TABLE 3

Consistency analysis of PRs for the carrier status considering children and sociodemographic characteristics, hygiene practices, and water treatment methods

Very high-risk water		aPR	95% CI	*P*-value
	No	Ref.		
Model A: non-aPR considering the very high-risk water	Yes	4.71	2.05–10.80	< 0.001
Model B: Model A adjusted with children characteristics (gender and chronic malnutrition)	Yes	4.40	1.94–9.98	< 0.001
Model C: Model B adjusted with sociodemographic (socioeconomic status and water source)	Yes	2.61	1.04–6.58	0.041
Model D: Model C adjusted with hygiene practices (daily washing and cleaning supplies for washing the storage container)	Yes	4.00	1.06–15.18	0.041
Model E: Model D adjusted with water treatment methods (drinking water boiling and chlorination)	Yes	3.82	1.13–12.89	0.031

aPR = adjusted prevalence ratio; PR = prevalence ratio.

Water sources in low- and middle-income countries are frequently contaminated.[2–4,17] Several studies suggest that increasing access to safe water could reduce the burden of diarrheal disease.[3,5,7,39,40] Universal access to continuous water service through domestic pipes is generally seen as the first step to avoid the use of potentially unsafe water sources. However, piped water, and other improved water sources do not necessarily provide safe water.[2,29,41,42] Our findings are consistent with other reports as we did not find significant differences in the microbial load of stored water in households with domestic pipes compared with those that access exclusively shared-use water sources.[2,41] However, it is important to consider that the contamination of water samples collected in this study could reflect contamination that occurred anywhere from the water source to the point of consumption and includes household hygiene practice effects, as previously suggested in India.[1] Studies to identify the more likely causes for this contamination are needed to better produce, protect, and deliver safe drinking water.

In this study, the daily washing of the container used to store drinking water was a risk factor associated with a higher prevalence of carriers. The use of bleach and water to wash the container was associated with a lower prevalence of carriers. However, after adjusting for water source and daily washing, the use of bleach was no longer a significant factor but was still nonsignificantly linked to a lower risk of carriage. It is important to highlight that cleaning supplies for washing and daily washing were self-reported by mothers/caregivers and not evaluated by direct observation, which may have resulted in overreporting of container cleaning. Despite this potential bias, these results suggest that the use of biocidal agents, such as bleach, should be promoted as a part of good hygiene practices for washing containers and surfaces.

Boiling and chlorination are methods to make water safe to drink[7,29]; however, a suboptimal practice may be ineffective in eliminating microorganisms.[43,44] Water-hygiene practices are associated with the availability of treatment supplies and the mother's knowledge of water treatment and waterborne diseases.[45,46] In this study, the reported frequencies of boiling and chlorine use were comparable with those described in another Peruvian community.[44] We did not find an association between reported treatment practices and carrier status. This lack of association could be explained by ineffective water treatments or by social desirability bias of each household having to directly report to one of the study's field workers. The verification of treatment by direct observation, measurements of free chlorine, or a comparison of the bacterial load in untreated and treated water are actions that could help to clarify the association between boiling and chlorination and the prevalence of those who asymptomatically carry diarrheal pathogens.

Drinking water is not the only vehicle or source of enteric pathogens. Other potential sources of contamination in regards to children may be intra-domiciliary contacts, contaminated pacifiers, or glasses, and the direct ingestion of soil or animal feces have been described elsewhere.[5,14,15,47–50] We evaluated samples from containers used to store the drinking water and not samples from domestic pipes or shared water sources. However, the high prevalence of children who tested positive for pathogens in households that used shared water sources should be considered as a warning signal that requires attention. It is challenging to elucidate correctly the association between water quality and pathogen carriage in this study because 1) both were measured in one point in time because of the cross-sectional design, 2) we did not confirm if children consumed the drinking water that was available at the study sampling day, and 3) covariates such as hygiene practices are susceptible to bias. In addition, our estimates do not account for the potential presence of geographic clustering within villages because we did not collect geolocation data from the study households, an issue that future studies can take into account. However, the drinking water was collected from the container that was usually used to store water consumed by the child and other people at home.

In conclusion, we found that the presence of very high-risk household drinking water was associated with a higher prevalence of children carrying pathogenic enteric bacteria. This association suggests that contaminated drinking water plays an important role in the generation of carriers and should be considered a threat to the health of the child and household. The promotion of good safety practices for handling and storing drinking water at home is needed to avoid exposures to risks. Further studies are needed to validate our results and better determine the risk of disease to the child and other household members. Evidence-based and appropriate public health measures to control infection with diarrheagenic pathogens are needed in rural communities of Peru.

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