JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Multidrug-resistant *Escherichia coli* and its role in stunting: evidence from a cross-sectional study in Indonesia

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Abstract

Introduction: This cross-sectional study aimed to investigate the relationship between environmental contamination and stunting among children under five years of age in Langkat, North Sumatra, Indonesia.

Methodology: This cross-sectional study was conducted from February to October 2024, involving 180 households with private drinking water sources. Stunting was assessed using height-for-age Z-scores (< -2 SD). Water samples were tested for *E. coli* and *Coliforms* using the membrane filtration method on Chromocult Coliform Agar, incubated at 37°C for 24 hours. *E. coli* was confirmed by IMViC biochemical tests, and antimicrobial resistance was assessed using the Kirby-Bauer disk diffusion method following CLSI guidelines.

Results: Water testing showed *E. coli* and Coliform bacteria in 75.4% and 80.7% of samples, indicating significant contamination. *E. coli* presence was significantly associated with stunting ($p = 0.044$), turbidity ($p = 0.047$), and diarrhea history ($p = 0.004$). Hierarchical regression identified *E. coli*, diarrhea, and turbidity as key stunting predictors, with *E. coli* showing a beta of -0.287 ($p = 0.035$). Multidrug resistance was high among *E. coli* isolates (n = 108), with 92.59% resistant to Tetracycline and Sulfamethoxazole, and 70.37% to Amoxicillin and Ampicillin, underscoring the need for targeted antimicrobial stewardship.

Conclusions: These findings highlight that environmental contamination, particularly from *Escherichia coli*, may represent a critical risk factor for stunting, emphasizing the need for improved water quality and hygiene practices in vulnerable communities.

Key words: *Coliforms*; diarrhea; multidrug resistance; stunting; water quality assessment.

J Infect Dev Ctries 2026; 20(5):660-669. doi:10.3855/jidc.21502

(Received 23 February 2025 – Accepted 12 November 2025)

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Introduction

Stunting remains a critical public health concern in Indonesia. According to the Indonesia Health Survey 2023, 21.5% of children under five nationwide are stunted. In North Sumatra, the prevalence reaches 18.9% among children under five and 14.8% among children under two, indicating a substantial burden even in early childhood [1]. These figures persist despite extensive national nutrition programs, suggesting that dietary interventions alone may be insufficient to address growth faltering [2,3].

Recent evidence has shifted attention toward chronic microbial exposure as a significant yet underrecognized pathway leading to impaired growth [4,5]. The WHO/UNICEF Joint Monitoring Programme estimates that 2.1 billion people globally lack access to safe drinking water, and 4.2 billion do not have access to basic sanitation [6]. Such deficits are disproportionately concentrated in rural settings, where only 60% of households have safely managed drinking water compared to 86% in urban areas [7]. Under these conditions, children are repeatedly exposed to enteric pathogens such as *Escherichia coli*, leading to environmental enteric dysfunction (EED), malabsorption, and ultimately growth impairment [8-11]. However, current understanding of the link between water microbiology and stunting in Indonesia remains fragmented, with limited integration of environmental and clinical data.

Compounding the issue is the emerging threat of antimicrobial resistance (AMR) among waterborne bacteria. A recent Indonesian report documented carbapenem resistance in 21.6% of *Klebsiella pneumoniae* and 18.3% *of E. coli* isolates in hospital settings, with community isolates showing even higher resistance rates (28.3% for *K. pneumoniae* and 15.7% for *E. coli*) [12]. Nationwide, bacterial AMR was estimated to contribute to 34,530 - 133,753 deaths in 2019 [13]. The presence of multidrug-resistant *E. coli* in household water supplies may prolong or exacerbate enteric infections, limit treatment efficacy, and sustain intestinal inflammation, yet this potential pathway to growth failure has rarely been explored in rural Indonesian populations.

Therefore, this observational cross-sectional study aimed to investigate the microbiological quality of

drinking water sources among stunted and non-stunted children in Langkat Regency, North Sumatra. Specifically, we isolated and characterized *E. coli* from water samples, assessed their antimicrobial resistance profiles, and explored associations between water contamination and stunting status. By integrating environmental bacterial data, this study provides novel insight into the potential contribution of waterborne AMR pathogens to the persistence of stunting in rural Indonesia.

Methodology

Study design and population

A cross-sectional study was conducted between February and July 2024 in Langkat Regency, North Sumatra Province, Indonesia (3°14'00" - 4°13'00" N; 97°52'00" - 98°45'00" E), comprising 23 sub-districts over 6,263 km^2. In 2023, the prevalence of stunting among children under five in this region was 18.9%. The minimum required sample size was calculated using the Lemeshow formula with 95% confidence and 0.30 relative precision, yielding 183 participants. Accounting for < 30% anticipated non-response, 171 children were randomly selected.

Ethical approval was granted by the Research Ethics Committee of Medan Health Polytechnic, Ministry of Health, Indonesia (KH.03.01/XI.8/1517/2024). Written informed consent was obtained from parents. Households were eligible if they used a private drinking water source (well or refillable water).

Stunting assessment and covariates

Stunting was defined as a height-for-age Z-score < −2 SD; ≥ −2 SD was classified as normal. Anthropometric measurements were conducted by trained local health workers. Sociodemographic and health-related variables were obtained through parent-completed questionnaires, maternal and child health records, and interviews. Variables included child age, gender, maternal education, family income, diarrhea in the past seven days, feeding practices, immunization status, and primary drinking water source.

Water quality variables included the presence of *Escherichia coli*, coliforms, pH, and turbidity. pH was measured in situ using a handheld pH meter, while turbidity was determined using nephelometry [14]. Water was deemed acceptable if the pH ranged between 6.5 and 7.5 according to Minister of Health Regulation No. 32/2017.

Water sampling and bacterial isolation

Water collection adhered to U.S. Environmental Protection Agency guidelines for microbiological water quality analysis [15]. Approximately 200 mL of water was collected from children's primary drinking sources in pre-sterilized 250 mL Erlenmeyer flasks. Samples were labelled, stored at 2–8 °C in insulated coolers, and transported to the laboratory within six hours.

For bacterial enumeration, 100 mL of each sample was filtered through a 0.45 μm, 47 mm sterile cellulose nitrate membrane using a vacuum filtration unit. Filters were placed on Chromocult Coliform Agar (CCA) and incubated at 36 ± 2 °C for 24 hours. *E. coli* colonies were identified by dark blue/violet pigmentation, while other coliforms appeared pink/red.

Quality assurance included sterile water negative controls to monitor for potential contamination during sampling or analysis, and duplicate sampling in 10% of households.

Verification and biochemical confirmation of E. coli

To confirm the identification of *E. coli* colonies, dark blue or violet-blue colonies observed on CCA media were carefully isolated using a sterile inoculating loop. These colonies were streaked onto Eosin Methylene Blue Agar (EMBA), which serves as a differential medium for *E. coli*. The plates were incubated at 37 °C for 18–24 hours. After incubation, colonies on EMBA were visually assessed. Characteristic *E. coli* colonies on EMBA should have a metallic green sheen.

Presumptive *E. coli* colonies exhibiting dark blue to violet pigmentation on CCA were further verified by streaking onto Eosin Methylene Blue Agar (EMBA) and incubating at 37 °C for 18–24 hours. Colonies displaying the characteristic metallic green sheen on EMBA were subjected to a series of confirmatory biochemical assays following standard microbiological procedures. Each isolate was inoculated onto Triple Sugar Iron Agar (TSIA) to assess glucose, lactose, and sucrose fermentation, as well as gas and hydrogen sulphide production; typical *E. coli* isolates produced an acid/acid (A/A) reaction, indicated by yellow coloration in both the slant and butt, often accompanied by gas formation without black precipitate. Indole production and motility were evaluated using Sulphur Indole Motility (SIM) medium, where a cherry-red surface layer after the addition of Kovac's reagent confirmed indole positivity, and diffuse growth away from the stab line indicated motility, with the absence of blackening signifying no H_2S production. Finally, citrate utilization was examined on Simmons Citrate Agar (SC), and

isolates retaining the original green colour were interpreted as citrate-negative, consistent with the metabolic profile of *E. coli*.

Antibiotic susceptibility testing

A total of 513 *E. coli* isolates (three per household) were tested using the Kirby–Bauer disk diffusion method on Mueller-Hinton Agar (MHA), following Clinical and Laboratory Standards Institute (CLSI) protocols [16]. Inocula were standardized to 0.5 McFarland (approximately 1.5×10^8 CFU/mL) using a DensiCHEK [17]. Antibiotic disks included Ampicillin (10 µg), Amoxicillin (10 µg), Ciprofloxacin (5 µg), Nalidixic Acid (30 µg), Cefotaxime (30 µg), Sulfamethoxazole (25 µg), and Tetracycline (30 µg). Plates were incubated at 37 °C for 16–18 hour, and inhibition zones were measured at five angles using a calliper [18]. Interpretation followed CLSI criteria [16,19].

Figure 1. Map of the sampling area of Langkat Regency, North Sumatra, Indonesia.

Geographical map of the sampling area in Langkat Regency, North Sumatra, Indonesia, highlighting the locations of 180 surveyed households (red dots) involved in a cross-sectional study on *Escherichia coli* contamination and stunting among children under five years of age. The map highlights sub-district boundaries, oil palm plantations, and geographical context relative to North Sumatra Province.

Data analysis

A descriptive statistical analysis was conducted to examine the sociodemographic characteristics of the target families. Associations between water quality predictors and stunting were analyzed using Pearson's chi-square test ($p < 0.05$). Linear regression was performed with Z-scores as continuous outcomes to estimate effect magnitude (R^2). Hierarchical regression models incorporated water quality, medical history, and water source variables, adjusting for age and gender as confounders.

Table 1. Social, demographic, and environmental profiles of child participants.

Characteristic	n (%)
Gender	
Female	87 (50.9)
Male	84 (49.1)
Age	
≤12 months	45 (26.3)
13-24 months	48 (28.1)
25-36 months	30 (17.5)
37-48 months	39 (22.8)
49-60 months	9 (5.3)
Mother's education	
Primary school – Junior high school	60 (35.1)
Senior high school- Bachelor degree	111 (64.9)
Family income	
≤ Rp. 1,500,000	27 (15.8)
Rp. 1,500,000 – 3,000,000	108 (63.2)
> 3,000,000	36 (21.1)
Weight	
6.0-9.9 kg	75 (43.9)
10.0–14.9 kg	75 (43.9)
15.0-19.9 kg	21 (12.3)
Height	
61-80 cm	75 (43.9)
81-100 cm	81 (47.4)
101-120 cm	15 (8.8)
***E. coli* presence**	
Present	129 (75.4)
Absent	42 (24.6)
***Coliform* presence**	
Present	138 (80.7)
Absent	33 (19.3)
Turbidity	
≤5 NTU	138 (80.7)
> 5 NTU	33 (19.3)
pH	
Neutral pH	84 (49.1)
Non-neutral pH	87 (50.9)
Repeated episodes of diarrhea within 3 months	
Yes	48 (28.1)
No	123 (71.9)
Drinking water source	
Well water	129 (75.4)
Refill water	42 (24.6)
The way a child is fed	
Hand	51 (29.8)
Utensils	72 (42.1)
Both	48 (28.1)
Immunization	
Complete	165 (96.5)
Incomplete	6 (3.5)

Results

Sociodemographic and environmental characteristics of the participants

A total of 171 children from ten locations in Langkat Regency were included in the analysis (Figure 1). Slightly more than half were female (50.9%), while 49.1% were male. Most participants were aged 13–24 months (28.1%), weighed 6.0–14.9 kg (87.8%), and had a height of 61–100 cm (91.3%). Maternal education was predominantly at senior high school or bachelor's level (64.9%), and 63.2% of households reported a monthly income between IDR 1,500,000 and IDR 3,000,000.

Environmental assessment revealed substantial water quality concerns. *Escherichia coli* contamination was detected in 75.4% of household water samples, while total Coliforms were present in 80.7%. Furthermore, 19.3% of samples exhibited turbidity levels > 6 NTU, and 50.9% failed to meet the recommended drinking water pH range. Despite this, only 28.1% of children experienced frequent diarrhea within the preceding seven days. Most households relied on well water (75.4%) as the primary source for child consumption. Feeding practices varied, with 42. 1% of caregivers using utensils and 29.8% using hands. Immunization coverage was notably high at 96.5%. These integrated findings (Table 1) highlight a paradoxical coexistence of high environmental contamination with relatively low reported morbidity.

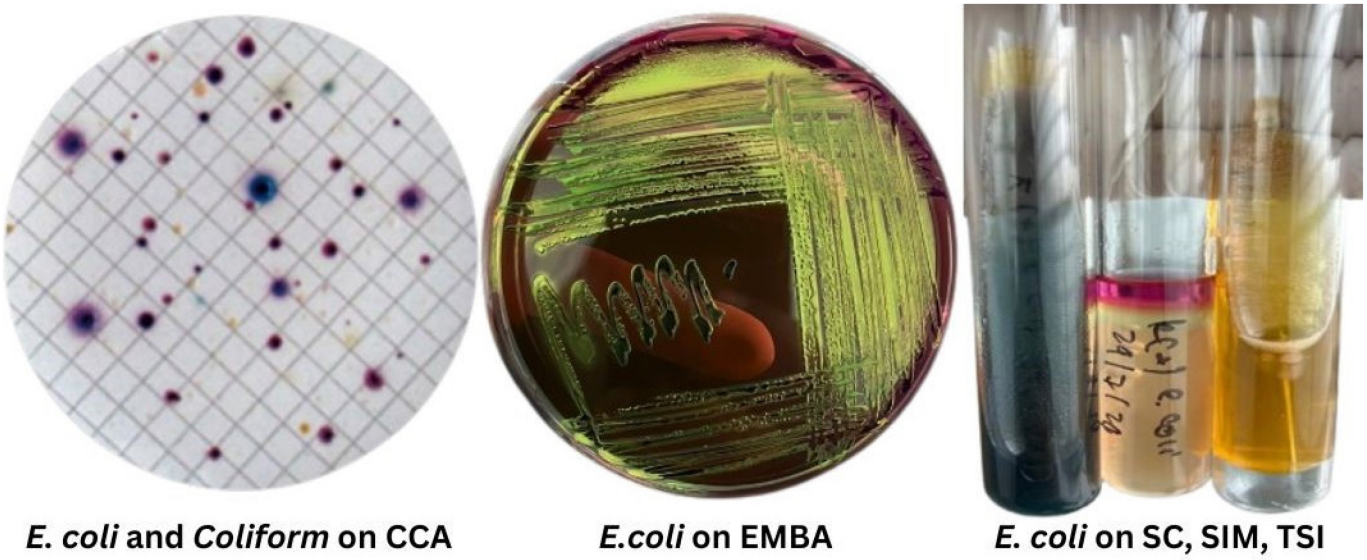
Bacterial isolation and confirmation

Selective and differential culture methods enabled clear differentiation between *Escherichia coli* and other coliforms. On Chromocult Coliform Agar (CCA), colonies displaying a dark blue to violet colour were classified as *E. coli*, whereas pink to red colonies represented non-E. coli coliforms (the left panel of Figure 2). Subsequent streaking on Eosin Methylene Blue Agar (EMBA) confirmed *E. coli* identity through characteristic metallic green sheen resulting from vigorous lactose fermentation. Biochemical verification further substantiated the identification (the right panel of Figure 2). On SIM medium, confirmed *E. coli* isolates exhibited diffuse motility and produced a red indole layer upon Kovac's reagent addition, with no blackening indicative of absent H_2S production. Lack of citrate utilization was confirmed by retention of green coloration on Simmons Citrate Agar. Triple Sugar Iron Agar reactions were consistently acid/acid (yellow slant and butt) with gas formation and no H_2S precipitate, reflecting fermentation of glucose and lactose/sucrose. Collectively, the congruence of chromogenic, selective, and biochemical profiles provided robust confirmation of *E. coli* presence in household water samples.

Multidrug resistance test of isolates

Figure 3 illustrates the antibiotic resistance profiles of *Escherichia coli* pathotypes (n = 513). The findings reveal significant resistance to commonly used

Figure 2. Isolation and verification of *E. coli* from a water sample by in vitro testing.



Isolation and identification of *Escherichia coli* from water samples using selective and differential culture media. (Left) Chromogenic Coliform Agar (CCA) showing *E. coli* as dark blue to violet colonies and other coliforms as pink to red colonies. (Center) Eosin Methylene Blue Agar (EMBA) displays a characteristic metallic green sheen, confirming *E. coli* presence. (Right) Biochemical verification using Sulphide-Indole-Motility (SIM), Simmons Citrate (SC), and Triple Sugar Iron (TSI) tests, indicating positive indole production, negative citrate utilization, and acid-gas fermentation, respectively. This in vitro analysis confirms the prevalence of *E. coli* contamination in drinking water, which is significantly associated with stunting in children under five years of age.

antibiotics, highlighting alarming trends. The highest resistance was observed against amoxicillin (56.14%) and ampicillin (50.68%), suggesting widespread inefficacy of these β-lactam antibiotics. In contrast, the lowest resistance was noted for ciprofloxacin (12.87%) and cefotaxime (14.42%), indicating their relative effectiveness against *E. coli*.

Moderate resistance rates were recorded for tetracycline (37.04%), nalidixic acid (28.27%), and sulfamethoxazole (21.25%), reflecting a concerning reduction in their therapeutic potential. These findings indicate the circulation of multidrug-resistant *E. coli* strains in domestic water supplies, posing a significant public health challenge. The data support the urgent need for targeted antibiotic stewardship programs to curb the development and spread of resistant strains.

Association between height-for-age index and risk factors

The distribution of height-for-age index (HAZ) across socio-demographic and environmental variables is presented in Table 2. The data indicate that the HAZ varies across different demographic and environmental factors. Chi-square analysis indicated no significant associations with gender (p = 0.923), age group (p = 0.390), maternal education, family income, water source, feeding practices, or immunization status. In contrast, *E. coli* contamination (p = 0.044), diarrhea incidence (p = 0.004), and turbidity levels (p = 0.047) were significantly associated with lower HAZ categories, suggesting a strong environmental component in the growth impairment.

Table 3 shows the results of a hierarchical regression analysis that looks at how different factors affect a specific health outcome in children. The

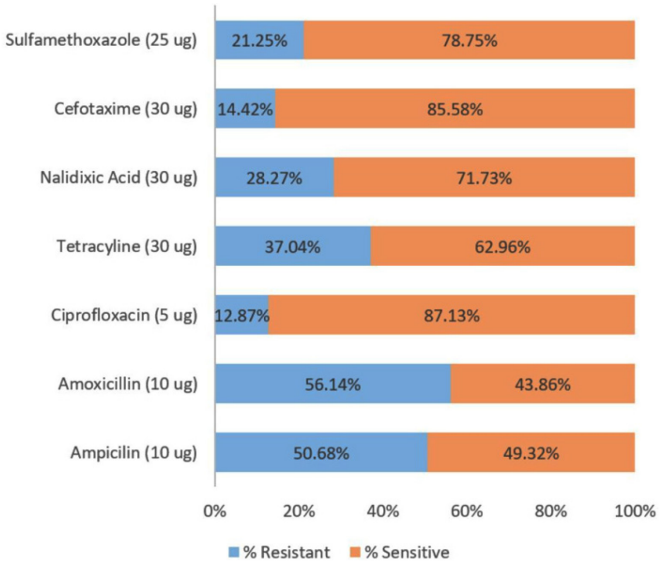
Table 2. Distribution of height-for-age index (HAZ) and associated factors.

	HAZ			Total	Person's Chi-Square (p)
	Z-score < -3.0	-3.0 ≥ Z-score < -2.0	Z-score ≥ -2.0		
Gender					
Female	39	15	33	87	0.923
Male	36	18	30	84	
Age					
≤12 months	18	6	21	45	
13-24 months	18	12	18	48	
25-36 months	12	3	15	30	0.390
37-48 months	24	6	9	39	
49-60 months	3	6	0	9	
Mother's education					
Primary school – Junior high school	27	9	24	60	0.824
Senior high school- Bachelor degree	48	24	39	111	
Family income					
≤ Rp. 1,500,000	9	0	18	27	
Rp. 1,500,000 – 3,000,000	48	30	30	108	0.086
> 3,000,000	18	3	15	36	
E. coli					
Present	45	27	57	129	0.044*
Absent	30	6	6	42	
Coliform					
Present	57	30	51	138	0.579
Absent	18	3	12	33	
Turbidity					
Clear	51	27	60	138	0.047*
Turbid	24	6	3	33	
pH					
Neutral pH	42	18	24	84	0.444
Non-neutral pH	33	15	39	87	
Diarrhea					
Present	15	0	33	48	0.004*
Absent	60	33	30	123	
Drinking water source					
Well water	57	30	42	129	0.760
Refill water	18	3	21	42	
The way a child is fed					
Hand	18	12	21	51	
Utensils	30	9	33	72	0.237
Both	27	12	9	48	
Immunization					
Complete	72	33	60	165	0.408
Incomplete	6	0	0	6	

analysis is divided into two models. Model 1 of the hierarchical regression included only gender and age, explaining 3.1% of the variance in the health outcome. Neither variable demonstrated a significant effect, with gender showing a beta coefficient of −0.041 ($p = 0.768$) and age a beta of 0.181 ($p = 0.194$), indicating that basic demographic characteristics alone do not substantially contribute to outcome variation.

Model 2 added environmental and behavioural variables, including *E. coli* and Coliform contamination, diarrhea history, turbidity, pH, feeding practices, and source of drinking water. In this extended model, *E. coli* contamination demonstrated a significant negative association ($\beta = -0.287$, $p = 0.035$), confirming its detrimental influence on child health. Coliform contamination did not show a meaningful effect ($\beta = -0.040$, $p = 0.785$). Diarrhea ($\beta = 0.310$, $p = 0.025$) and turbidity ($\beta = -0.286$, $p = 0.036$) emerged as statistically significant predictors; however, the direction of association for diarrhea and turbidity should be interpreted cautiously due to the potential for confounding or reverse causality. Overall, Model 2 explained 30.0% of the variance, indicating that environmental exposures, particularly *E. coli* contamination, played a substantially larger role than individual demographics in determining children's nutritional status (as reflected by height-for-age Z-scores).

Figure 3. Antibiotic resistance profiles of *E. coli* pathotypes (n = 513).



Antibiotic resistance profiles of *Escherichia coli* isolates (n = 513) from drinking water samples in Langkat Regency, North Sumatra, Indonesia. The highest resistance rates were observed for amoxicillin (56.14%) and ampicillin (50.68%), followed by tetracycline (37.04%) and nalidixic acid (28.27%), indicating widespread β-lactam and tetracycline resistance. Lower resistance was detected against sulfamethoxazole (21.25%), cefotaxime (14.42%), and ciprofloxacin (12.87%), suggesting partial efficacy of third-generation cephalosporins and fluoroquinolones. These findings emphasize the urgent need for antimicrobial stewardship to mitigate the public health risk posed by multidrug-resistant *E. coli* in vulnerable communities.

Discussion

The findings of this study emphasize the critical relationship between environmental factors, particularly water contamination, and children's nutritional status. By focusing on microbial contamination, particularly *Escherichia coli* and *coliform* bacteria, this study provides further insight into their potential role in childhood stunting. The detection of *E. coli* in 75.4% and coliforms in 80.7% of household water samples underscores the significant impact of inadequate WASH (Water, Sanitation, and Hygiene) conditions on child growth. This aligns with previous studies in low- and middle-income countries, where *E. coli* was found in 70% of rural drinking water in northwestern Ethiopia and 56.2% in rural southwestern Uganda [20,21].

The presence of *E. coli* in drinking water serves not only as a microbiological indicator of fecal contamination but also as a plausible environmental risk factor for stunting, given its established role in promoting environmental enteric dysfunction (EED), recurrent diarrhea, and impaired nutrient absorption [22-25]. In this study, *E. coli* contamination in

Table 3. Hierarchical regression analysis of sociodemographic and water contamination variables affecting stunting in children.

Model	Variable	Beta Coefficients	Sig. Value	95% Confidence interval for B		R^2 for model	*p*
				Lower bound	Upper bound		
Model 1	Gender	-0.041	0.768	-0.568	0.422	0.031	0.427
	Age	0.181	0.194	-0.068	0.329		
Model 2	Gender	-0.010	0.938	-0.486	0.450	0.300	0.036*
	Age	-0.048	0.738	-0.240	0.171		
	E. coli contamination	-0.287	0.035*	-0.758	0.576		
	Coliform contamination	-0.040	0.785	-1.152	-0.043		
	Diarrhea	0.310	0.025*	0.080	1.157		
	Turbidity	-0.286	0.036*	-0.045	1.252		
	pH	-0.125	0.340	-0.689	0.243		
	The way a child is feed	0.096	0.479	-0.317	0.666		
	Water drinking source	0.047	0.715	-0.435	0.629		

household water sources showed a statistically significant association with stunting incidence (p = 0.044), reinforcing previous findings from similar settings. Several lines of evidence from previous literature strengthen the plausibility of *E. coli* contamination as a causal pathway linking poor water quality to impaired child growth. A large-scale analysis in Bangladesh demonstrated that exposure to highly contaminated drinking water was associated with up to a 9 percentage points increase in stunting risk, even after adjusting for socioeconomic confounders, indicating that *E. coli* exposure at the household level is an independent predictor of linear growth failure [24]. This epidemiological finding is biologically coherent with experimental and clinical data showing that chronic ingestion of enteric pathogens, particularly pathogenic *E. coli* strains, induces EED, a subclinical inflammatory condition characterized by villous atrophy, increased intestinal permeability, and reduced absorptive surface area [26-28]. At the level of specific pathogens, several in vitro studies on Enteroaggregative *Escherichia coli* (EAEC) strains have demonstrated that bacterial–epithelial interactions, including adhesion, EGFR signalling activation, and IL-8 secretion, trigger sustained intestinal inflammatory responses. Such mechanisms are biologically consistent with the pathway of EED and provide a plausible explanation for how exposure to pathogenic *E. coli* contributes to impaired nutrient absorption and growth faltering [29]. These mechanistic insights, taken together with epidemiological associations observed in multiple settings, provide a coherent biological rationale for linking *E. coli* exposure to impaired growth. Nevertheless, it is important to distinguish biological plausibility from confirmed causality. Although causality cannot be fully inferred due to the cross-sectional design, the consistent epidemiological evidence and strong biological plausibility underscore *E. coli* exposure as an environmental exposure biomarker and a credible pathway linking poor WASH conditions to linear growth faltering.

In terms of water quality, 19.3% of clean water samples exceeded turbidity thresholds, and 50.9% failed to meet pH standards. Importantly, turbidity showed an inverse association with child growth (p = 0.047), underscoring its utility as a physical indicator of water hygiene. Turbidity reflects the presence of particulate matter and microbial contaminants, making it a critical parameter for WASH interventions [28-30]. These findings highlight the urgent need for targeted measures to improve water safety and reduce stunting in the study region. Although we identified significant associations, particularly between *Escherichia coli* presence, water turbidity, and stunting, the cross-sectional design precludes establishing causality, as temporality remains unconfirmed and potential confounders cannot be fully ruled out.

A key strength of this research lies in its focus on environmental drivers of stunting, shifting attention from traditional sociodemographic factors. Unlike maternal education or family income, which showed no significant association with height-for-age indexin this study, environmental exposures, particularly bacterial contamination and poor water quality, emerged as dominant determinants. This finding is particularly relevant for rural areas where waterborne diseases are prevalent and access to clean water remains a challenge [31].

One notable observation from the analysis was the observed positive correlation between diarrhea incidence and HAZ (p = 0.025). While diarrhea is typically considered a detrimental health outcome, this result may reflect compensatory mechanisms in children with recurrent episodes or improved access to medical care in response to diarrheal illnesses. This aligns with hypotheses proposed by Goldberg *et al.* [32] but warrants further investigation to clarify the long-term developmental impacts of recurrent diarrhea.

A comparison of antimicrobial resistance (AMR) patterns between our findings and the meta-analysis by [33] provides valuable insights into regional variations in resistance trends and antibiotic stewardship. Our study demonstrates comparatively lower resistance rates for key antibiotics, including amoxicillin (56.14% vs. 80.9%) and ampicillin (50.68% vs. 73.5%), suggesting more effective stewardship or reduced selective pressure in our study region. Ciprofloxacin and cefotaxime remain particularly effective in our data (resistance rates of 12.87% and 14.42%, respectively), contrasting with the higher resistance rates reported by Salleh *et al.* (25.7% and 36.6%). Notably, lower resistance levels for tetracycline (37.04% vs. 54.7%) and nalidixic acid (28.27% vs. 58.2%) further highlight regional nuances in resistance development. These findings underscore the dynamic nature of AMR evolution and emphasize the critical need for region-specific antimicrobial stewardship strategies to address local resistance patterns and mitigate the global AMR crisis.

This study suggests that water contamination, particularly with *Escherichia coli* and *coliform* bacteria, may represent a critical risk factor for adverse child health outcomes, including stunting. The high prevalence of *E. coli* in household water samples

(75.4%) aligns with findings from prior studies, such as [34] and [35], which highlighted untreated well water as a common source of pathogenic microorganisms in rural areas. Notably, while we identified a novel correlation between water turbidity and children's height-for-age index, despite previous reports indicating no direct link between turbidity and growth metrics [36], we acknowledge that this finding may be limited by measurement or sampling constraints, such as turbidity coinciding with mineral-rich or treated water sources, seasonal variability, or unmeasured household water-use and handling behaviors, underscoring the need for longitudinal studies with detailed data on water treatment, healthcare access, and dietary intake to better clarify these relationships.

The identification of *E. coli* in this study utilized a combination of selective and differential microbiological techniques to ensure accuracy and reliability. Chromogenic Coliform Agar (CCA) effectively differentiated *E. coli* from other *coliforms* by producing distinct blue to purple colonies, while Eosin Methylene Blue Agar (EMBA) confirmed the species with its characteristic metallic green sheen. These widely recognized methods provide robust evidence for *E. coli* identification. Biochemical assays further validated the findings, with the Sulphur-Indole-Motility (SIM) test confirming motility and indole production, Simmons Citrate Agar (SC) demonstrating the inability to utilize citrate, and Triple Sugar Iron Agar (TSIA) revealing acid fermentation of glucose, lactose, and sucrose without hydrogen sulphide production. These results, consistent with the established characteristics of *E. coli* [37], provide strong microbiological confirmation and enhance the credibility of the study's conclusions.

By integrating microbial and physicochemical water quality assessments, this research underscores the multifaceted impact of water contamination on child health. The findings not only reaffirm the significance of *E. coli* as a key biomarker for waterborne disease risk but also highlight the potential of turbidity as an additional indicator of water quality's influence on growth outcomes. This study contributes valuable evidence to the ongoing effort to address stunting through targeted interventions in water safety and sanitation practices.

Conclusions

This study contributes to the existing literature by providing a comprehensive analysis of how environmental contamination, particularly waterborne pathogens, directly impacts child health outcomes in rural settings. The findings suggest that improving water quality and sanitation should be prioritized in public health interventions to mitigate the risks of stunting and other growth-related issues. This research also calls attention to the emerging challenge of antibiotic resistance in low-resource areas, underscoring the need for integrated approaches to address both environmental and microbial threats to child health. Future research could expand on these results by exploring interventions aimed at improving water quality in affected communities. Specifically, studies could investigate the impact of different water treatment methods in reducing *E. coli* contamination and their subsequent effects on child health and growth. Additionally, further genetic characterization of *E. coli* isolates would provide valuable insights into the prevalence of multidrug resistant (MDR) strains. Understanding resistance patterns, particularly in rural areas, could inform public health policies and antibiotic stewardship programs, contributing to the prevention of future health risks associated with *E. coli* infections.

Acknowledgements

We thank the Head of Health Service of Langkat Regency, North Sumatra, Indonesia, for granting permission for research in the area. Special thanks to Sawit Seberang and Seirapit Health Center, Langkat Regency, North Sumatra, Indonesia, for their technical support in the field.

Authors' Contributions

Method development, study design, and manuscript preparation: FS; Data collection, analysis, interpretation: TRS; Proofreading, practical supervision, validation: GSN, DS; Conceptualization and review: HIS.

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Conflict of interest

No conflict of interest is declared.

References

1. Ministry of Health (2023) The 2023 Indonesian Health Survey. Available: https://www.badankebijakan.kemkes.go.id/ski-2023-dalam-angka. Accessed: 19 September 2024.

2. Stewart CP, Iannotti L, Dewey KG, Michaelsen KF, Onyango AW (2013) Contextualising complementary feeding in a broader framework for stunting prevention. Matern Child Nutr 2: 27-45. doi: 10.1111/mcn.12088.
3. Schmidt CW (2014) Beyond malnutrition: The role of sanitation in stunted growth. Environ Health Perspect 122: 298-303. doi: 10.1289/ehp.122-A298.
4. Prendergast AJ, Humphrey JH (2015) Stunting persists despite optimal feeding: are toilets part of the solution? Nestlé Nutr Inst Workshop Ser 81: 99–110. doi: 10.1159/000365807.
5. Tjandrawinata RR, Amalia N, Tandi YYP, Athallah AF, Wibowo CA, Aditya MR, Muhammad AR, Azizah MR, Humardani FM, Nojaid A, Christabel JA, Agnuristyaningrum A, Nurkolis F (2025) The forgotten link: how the oral microbiome shapes childhood growth and development. Front Oral Health 6: 1-17. doi: 10.3389/froh.2025.1547099.
6. WHO/UNICEF (2021) Joint monitoring programme for water supply, sanitation and hygiene (JMP) Available: https: //washdata.org/report/jmp-2021-annual-report. Accessed: 19 September 2024.
7. Bolatova Z, Sharapatova R, Kanagat K, Kabiyev Y, Berndtsson R, Tussupova K (2025) Household satisfaction and drinking water quality in rural areas: a comparison with official access data. Sustainability 17: 1-12. doi: 10.3390/su17157107.
8. Tickell KD, Atlas HE, Walson JL (2019) Environmental enteric dysfunction: A review of potential mechanisms, consequences and management strategies. BMC Med 17: 181-192. doi: 10.1186/s12916-019-1417-3.
9. Mills K, Tan J, Guneratne T, Keir L, Goldstein M, Ventola C, Makama M, Ammerdorffer A, Gülmezoglu AM, Vogel JP, McDougall ARA (2025) Maternal gut microbiome interventions to improve maternal and perinatal health outcomes: Target product profile expert consensus and pipeline analysis. PLoS One 20: 1-17. doi: 10.1371/journal.pone.0321543.
10. Barlet LA, Bolick DT, Guerrant RL (2019) Disentangling microbial mediators of malnutrition: modeling environmental enteric dysfunction. Gastroenterol Hepatol 7: 692-707. doi: 10.1016/j.jcmgh.2018.12.006.
11. Rachmadi RA, Ariani Y, Alatas FS (2024) Impact of nutritional supplementation on environmental enteric dysfunction (EED) in children living in rural areas: a systematic review. Arq Gastroenterol 61: 1-10. doi: 10.1590/S0004-2803.24612023-159.
12. Gach MW, Lazarus G, Simadibrata DM, Sinto R, Saharman YR, Limato R, Nelwan EJ, Doorn HRV, Karuniawati A, Hamers RL (2024) Antimicrobial resistance among common bacterial pathogens in Indonesia: a systematic review. Lancet Reg Health - Southeast Asia 26: 1-14. doi: 10.1016/j.lansea.2024.100414.
13. Institute for Health Metrics and Evaluation (2019) Measuring infectious causes and resistance outcomes for burden estimation. Available: https:// vizhub.healthdata.org/microbe/. Accessed: 19 September 2024.
14. Matos T, Martins MS, Henriques R, Goncalves LM (2024) A review of methods and instruments to monitor turbidity and suspended sediment concentration. J Water Process Engineer 64: 1-10. doi: 10.1016/j.jwpe.2024.105624.
15. EPA (2023) Documentation for greenhouse gas emission and energy factors used in the waste reduction model (warm). United States Environ Protect Agency 16: 1-62. Available: https://www.epa.gov/system/files/documents/2023-12/warm-background_v16_dec.pdf. Accessed: 19 September 2024.
16. Clinical and Laboratory Standards Institute (CLSI) (2020) Performance Standards for Antimicrobial Susceptibility Testing. 30th Edition. Available: https://www.nih.org.pk/wp-content/uploads/2021/02/CLSI-2020.pdf. Accessed: 25 September 2024.
17. McFarland AJ, Grant GD, Perkins AV, Flegg C, Davey AK, Allsopp TJ, Renshaw G, Kavanagh J, McDermott CM, Anoopkumar-Dukie S (2013) Paradoxical role of 3-methyladenine in pyocyanin-induced toxicity in 1321N1 astrocytoma and SH-SY5Y neuroblastoma cells. Intern J Toxicol 32: 209–218. doi: 10.1177/1091581813482146.
18. Sufa HI, Kurniati I, Dermawan A, Abror YK, Indra AIN, Purkon DB (2024) Therapeutic potential of multi-targeting phytochemicals derived from *Apium graveolens* ethanol extract in West Java, Indonesia against multidrug-resistant Pseudomonas aeruginosa. Biodiversitas 25: 2183-2190. doi: 10.13057/biodiv/d250536.
19. Abu-Sini M, Maharmah RA, Abulebdah DH, Al-Sabi MN (2023) Isolation and identification of Coliform bacteria and multidrug-resistant *Escherichia coli* from water intended for drug compounding in community pharmacies in Jordan. Healthc 11: 299-310. doi: 10.3390/healthcare11030299.
20. Gizaw Z, Yalew AW, Bitew BD, Lee J, Bisesi M (2022) Stunting among children aged 24–59 months and associations with sanitation, enteric infections, and environmental enteric dysfunction in Rural Northwest Ethiopia. Sci Rep 12: 1-11. doi: 10.1038/s41598-022-23981-5
21. Lauer JM, Duggan CP, Ausman LM, Griffiths JK, Webb P, Bashaasha B, Agaba E, Turyashemererwa FM, Ghosh S (2018) Unsafe drinking water is associated with environmental enteric dysfunction and poor growth outcomes in young children in Rural Southwestern Uganda. Am J Trop Med Hyg 99: 1606-1612. doi: 10.4269/ajtmh.18-0143.
22. Hanif A, Nakano Y, Matsushima M (2024) The benefit of clean water on child health: an empirical analysis with specific reference to *Escherichia coli* water contamination. Water Resour Econ 47: 1-12. doi: 10.1016/j.wre.2024.100249.
23. Luby SP, Barch D, Whalen D, Tillman R, Belden A (2017) Association between early life adversity and risk for poor emotional and physical health in adolescence. JAMA Pediatr 171: 1168-1175. doi: 10.1001/jamapediatrics.2017.300.
24. Haque SS, Hoo BYR, Joseph G, Moqueet NS (2019) Children need clean water to grow: *E. Coli* contamination of drinking water and childhood nutrition in Bangladesh. Available: http://documents.worldbank.org/curated/en/542051573134521739. Accessed: 25 September 2024.
25. Sophie B, Alison HP, Paul TH, Camila G (2019) Environmental enteric dysfunction and child stunting. Nutr Rev 77: 240–253. doi: 10.1093/nutrit/nuy068.
26. Sari TR, Habib DA, Sembiring F, Nasution GS, Rahmah L (2023) Identification of *Escherichia coli*, *Shigella* sp., and *Staphylococcus aureus* as biomarkers of contamination in water sources in a stunting area of Belawan Sicanang Village, Medan Belawan District, Medan. J Clin Health Anal Sci 11: 225-234. doi:10.36341/klinikal_sains.v11i2.3892. [Article in Bahasa]
27. Tazi A, Araujo JR, Mulet C, Arena ET, Nigro G, Pedron T, Sansonetti PJ (2018) Disentangling host-microbiota regulation of lipid secretion by enterocytes: insights from commensals *Lactobacillus par*acasei and *Escherichia coli*. mBio 9: 1-18. doi: 10.1128/mBio.01493-18.
28. Vonaesch P, Araújo JR, Gody JC, Mbecko JR, Sanke H, Andrianonimiadana L (2022) Stunted children display ectopic

small intestinal colonization by oral bacteria, which cause lipid malabsorption in experimental models. Proc Natl Acad Sci 119: 1-12. doi: 10.1073/pnas.2209589119.
29. Archana J, Shipra C, Sujata G (2023) Enteroaggregative *Escherichia coli* induced activation of epidermal growth factor receptor contributes to IL-8 secretion by cultured human intestinal epithelial cells. Microbes Infec 25:1-15. doi: 10.1016/j.micinf.2023.105166.
30. Afiatun E, Wahyuni S, Supendi NI (2023) Evaluation of sanitation hygiene implementation at refill drinking water depot in Lembursitu Sub-District, Sukabumi City. IOP Conf. Ser.: Earth Environ Sci 1263: 1-7. doi: 10.1088/1755-1315/1263/1/012007.
31. Alvarez VF, Salazar DG, Figueroa C, Corrales JC, Casanova JF (2023) Estimation of water turbidity in drinking water treatment plants using machine learning based on water and meteorological data. Environ Sci Proc 25: 89-96. doi: 10.3390/ECWS-7-14326.
32. Konkobo FA, Diao M, Savadogo PW, Dakuyo R, Roamba NE, Zongo S, Dicko MH (2024) Reduction of malnutrition related to unsafe water consumption in developing countries: potabilization of surface water and traditional well water, with plant extracts. Int J Environ Res Pub Health 21: 519-526. doi: 10.3390/ijerph21050519.
33. WHO (2020) Malnutrition. Available: https://www.who.int/health-topics/malnutrition#tab=tab_1. Accessed: 20 September 2024.
34. Goldberg SB, Lam SU, Britton WB, Davidson RJ (2022) Prevalence of meditation-related adverse effects in a population-based sample in the United States. Psychother Res 32: 291-305. doi: 10.1080/10503307.2021.1933646.
35. Saleh MZ, Zuraina NMNN, Hajissa K, Ilias MI, Deris ZZ (2022) Prevalence of multidrug-resistant diarrheagenic *Escherichia coli* in Asia: a systematic review and meta-analysis. Antibiotics 11: 1-21. doi: 10.3390/antibiotics11101333.
36. Dillon J, Amaya J, Miller AJ, Xie SP, Kosaka Y (2020) Physical drivers of the summer 2019 North Pacific marine heatwave. Nat Commun 11: 1-11. doi: 10.1038/s41467-020-15820-w.
37. Wright CJ, Sargeant JM, Edge VL, Ford JD, Farahbakhsh K, Shiwak I, Flowers C, Harper SL (2018) Water quality and health in northern Canada: stored drinking water and acute gastrointestinal illness in Labrador Inuit. Environ Sci Pollut Res 25: 32975–32987. doi: 10.1007/s11356-017-9695-9
38. Feng P, Weagant SD, Grant MA, Burkhardt W (2020) Enumeration of *Escherichia coli* and the coliform bacteria. Bacteriological Analytical Manual (BAM). Available: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-4-enumeration-escherichia-coli-and-coliform-bacteria. Accessed: 19 September 2024.