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Microbiological quality of inland and coastal springs: *Escherichia coli*, other coliforms, and implications for public health

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Abstract

Untreated springs remain important domestic water sources in rural communities, yet their microbial safety and spatial and seasonal variability are insufficiently characterised. This study evaluated concentrations of *Escherichia coli* and other coliforms in inland and coastal springs within the King Sabata Dalindyebo Local Municipality, South Africa. Ten springs, five inland and five coastal, were sampled on five occasions during both wet and dry seasons, producing 100 water samples. Microorganisms were enumerated by membrane filtration, and concentrations were expressed as colony-forming units per 100 mL (CFU/100 mL). Separate negative binomial generalised linear mixed-effects models were fitted for each microbial indicator, with region and season as fixed effects and spring identity as a random intercept to account for repeated observations. *E. coli* was detected in all samples at concentrations ranging from 4 to 470 CFU/100 mL. After adjustment for region and repeated observations within springs, expected *E. coli* concentrations were 38.6% lower during the wet season than during the dry season (incidence rate ratio [IRR] = 0.614, 95% confidence interval [CI]: 0.468–0.805, $p < 0.001$). The adjusted inland–coastal difference in *E. coli* was not statistically significant (IRR = 1.346, 95% CI: 0.912–1.987, $p = 0.135$). Expected other-coliform concentrations were 19.3% lower during the wet season (IRR = 0.807, 95% CI: 0.720–0.905, $p < 0.001$) and 23.4% higher in inland springs compared with coastal springs (IRR = 1.234, 95% CI: 1.019–1.494, $p = 0.031$). None of the samples complied with the South African National Standard 241-1:2015 requirement for *E. coli* to be undetectable in 100 mL of drinking water. The findings demonstrate persistent faecal contamination across the investigated springs, with seasonal variation showing a more consistent association with microbial concentrations than broad inland–coastal classification. Routine microbial monitoring, improved spring protection, and effective household water treatment are required to reduce exposure to *E. coli* in communities that depend on untreated spring water.

Keywords: Faecal indicators; Membrane filtration; Rural sanitation; Source protection

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1. Introduction

A spring is a natural point at which groundwater flows onto the land surface, commonly where the water table or a pressurised aquifer intersects the ground. The resulting discharge may form a visible pool or stream, depending on the aquifer's geological and hydraulic characteristics.[1,2] Springs provide water for drinking, cooking, cleaning, and personal hygiene, as well as for livestock watering, irrigation, and small-scale farming.[3,4] They are particularly important in rural areas where centralised water-supply systems are absent, inadequate, or unreliable.[5] Springs also perform important ecological functions by sustaining river baseflows, providing habitats for local wildlife, and contributing to broader ecosystem health.[4,5]

The suitability of spring water for domestic use is determined by its physical, chemical, and microbiological characteristics. Among these, microbiological quality remains a critical determinant of drinking-water safety because faecal contamination may present an immediate risk to human health.[6,7] This study therefore focuses specifically on E*scherichia coli* and other coliforms as indicators of the microbiological quality of untreated spring water.

Despite global progress in expanding access to improved water supplies, many people continue to rely on water sources that lack adequate protection and routine water-quality monitoring. By 2020, approximately two billion people globally still lacked access to safely managed drinking water services, with the greatest inequalities occurring in rural communities in sub-Saharan Africa and parts of Asia.[8] Consequently, many rural households remain dependent on untreated natural water sources, including springs, wells, and surface water, which may expose users to microbiological contamination.

Evidence indicates that rural natural water sources are particularly vulnerable to faecal contamination.[9,10] A systematic review of 319 studies covering 96,737 water samples found that water sources in rural areas had 2.37 times higher odds of faecal contamination than those in urban areas. Improved sources had substantially lower odds of contamination than unimproved sources, although improved classification did not consistently guarantee microbiological safety.[9] This disparity may partly reflect dependence on unprotected or insufficiently monitored natural water sources situated near sanitation facilities, livestock areas, and other potential contamination pathways.

Microbial contamination primarily results from the introduction of human or animal faecal matter into a water source.[9,10] Potential pathways include inadequate sanitation, open defecation, livestock activity, wastewater disposal, poor waste management practices, and the movement of contaminated runoff into water sources.[11-13] In rural Lesotho, Gwimbi *et al.*[14] detected *E. coli* in all sampled drinking-water sources and reported significantly higher contamination in unprotected sources. Contamination was associated with inadequate source protection, open defecation, unhygienic practices, and the presence of livestock faeces and latrines near water sources.[14] These findings demonstrate how inadequate source protection and surrounding sanitation conditions may increase the vulnerability of untreated natural water sources.

E. coli is widely used as an indicator of recent faecal contamination and the possible presence of enteric pathogens in drinking water.[7,15] Other coliform bacteria may originate from faecal and non-faecal environments, including soil, vegetation, and natural water. Consequently, their detection is less specific for recent faecal contamination than the detection of *E. coli*. Nevertheless, their presence can provide a general indication of the microbial condition of a water source and its vulnerability to environmental contamination.[16,17] In contrast, detecting *E. coli* in drinking water constitutes a direct microbiological safety concern because some pathogenic strains can cause illnesses ranging from diarrhoea to severe infections.[18,19] High concentrations of microbial indicators therefore demonstrate the need for water-quality monitoring and appropriate public-health interventions.[12]

Studies from South Africa have similarly demonstrated the vulnerability of untreated natural water sources. In selected rural communities of the Eastern Cape Province, Zamxaka *et al.*[20] reported microbiological and physicochemical concerns in domestic water sources used by local households. In Limpopo Province, Edokpayi *et al.*[21] found that natural water sources used by rural communities were contaminated with *E. coli* and other coliform bacteria during both wet and dry seasons. These studies demonstrate that dependence on untreated natural sources may expose rural communities to persistent microbial risks.

Evidence from sub-Saharan Africa further illustrates how sanitation and groundwater conditions may influence natural water sources. Awuah[22] investigated 15 untreated wells in Ghana and found that microbial contamination was associated with pit-latrine depth and groundwater level. Although the study examined wells rather than springs, its findings demonstrate how interactions between sanitation conditions and local hydrogeology may compromise natural groundwater sources.

Microbial contamination of springs may vary spatially and seasonally. Rainfall can mobilise faecal material and

transport microorganisms into springs through surface runoff and rapid groundwater recharge.[23,24] However, microbial concentrations do not necessarily increase uniformly during wet periods because seasonal patterns may also be influenced by dilution, groundwater residence time, spring discharge, and local contamination.[25,26] Spatial variation may similarly reflect differences in hydrogeology, surrounding land use, source protection, and the intensity of human or animal activity. Repeated sampling across wet and dry periods and contrasting geographic settings is therefore necessary to characterise microbial variability reliably.[27-30]

Despite evidence of microbial contamination in rural natural water sources, limited research has compared the microbial condition of inland and coastal springs across wet and dry seasons within the same South African municipality. Previous studies have generally examined mixed domestic water sources, such as wells and other groundwater systems, without explicitly evaluating repeated seasonal measurements in inland and coastal spring environments.[20,22,24,26,27] Consequently, limited evidence is available on whether microbial concentrations vary by geographic setting and season in rural spring systems.

This study addresses this knowledge gap by assessing *E. coli* and other coliform concentrations in five inland and five coastal springs in King Sabata Dalindyebo Local Municipality, Eastern Cape Province. Each spring was sampled five times during the wet season and five times during the dry season, yielding 100 water samples. The study aimed to determine and compare microbial concentrations between inland and coastal springs and between wet and dry seasons. Its novelty lies in combining an inland–coastal comparison with repeated seasonal sampling and applying negative binomial mixed-effects models that account for repeated observations within individual springs. The findings provide locally specific evidence to support microbial monitoring, spring-source protection, and public health interventions in rural spring-dependent communities.

2. Methodology

2.1. Study area

The study was conducted in the King Sabata Dalindyebo Local Municipality, one of the five local municipalities within the OR Tambo District Municipality in the Eastern Cape Province of South Africa (Figure 1). The study area included inland and coastal rural settings. Coastal springs were located within 5 km of the coastline, whereas inland springs were located at least 20 km from it. The inland study sites included rural areas surrounding Mthatha and Qunu, while the coastal sites included areas surrounding Mqanduli, Coffee Bay, and Hole in the Wall. Rural settlements, subsistence farming, and livestock production occur within both geographic settings.

2.2. Sampling design

A purposive, spatially stratified sampling design was used to select springs from the inland and coastal regions of the municipality. In this study, 10 physical springs were selected, comprising 5 inland and 5 coastal springs. Each spring was assigned a permanent identification code that was retained throughout the study.

To examine seasonal variation, each of the 10 springs was sampled 5 times during the dry season and 5 times during the wet season, yielding 10 observations per spring. The total number of water samples was calculated using **Equation 1**:

$$N = 10\ springs \times 2\ seasons \times 5\ samples\ per\ spring\ per\ season = 100\ samples \quad (1)$$

Each seasonal group contained 50 observations, as did each regional group. These observations represent repeated sampling events rather than independent springs.

Figure 2 illustrates the distribution of the ten purposively selected springs across inland and coastal hydrogeomorphic zones within the King Sabata Dalindyebo Local Municipality. Inland springs (≥20 km from the coastline) and coastal springs (≤5 km from the shoreline) are displayed in separate panels for clarity. Each coloured point represents one of the five measurements collected per spring in both the wet and dry seasons

2.3. Sample collection and transportation

Spring-water samples were collected aseptically in sterile 1-L high-density polyethylene containers. A separate sterile container was used for each spring during every sampling event. Immediately after collection, the containers were sealed and labelled with the permanent spring identification code, sampling date, season, and region.

Samples were stored upright in a cooler box containing ice packs immediately after collection. The transportation temperature was maintained below 10 °C, and the samples were delivered to the laboratory and analysed within four hours of collection to minimise microbial die-off or regrowth in accordance with the procedures described previously.[31]

2.4. Membrane filtration and microbial enumeration

E. coli and other coliforms were enumerated using the membrane-filtration method.[31] The reusable filtration apparatus was sterilised before analysis and between

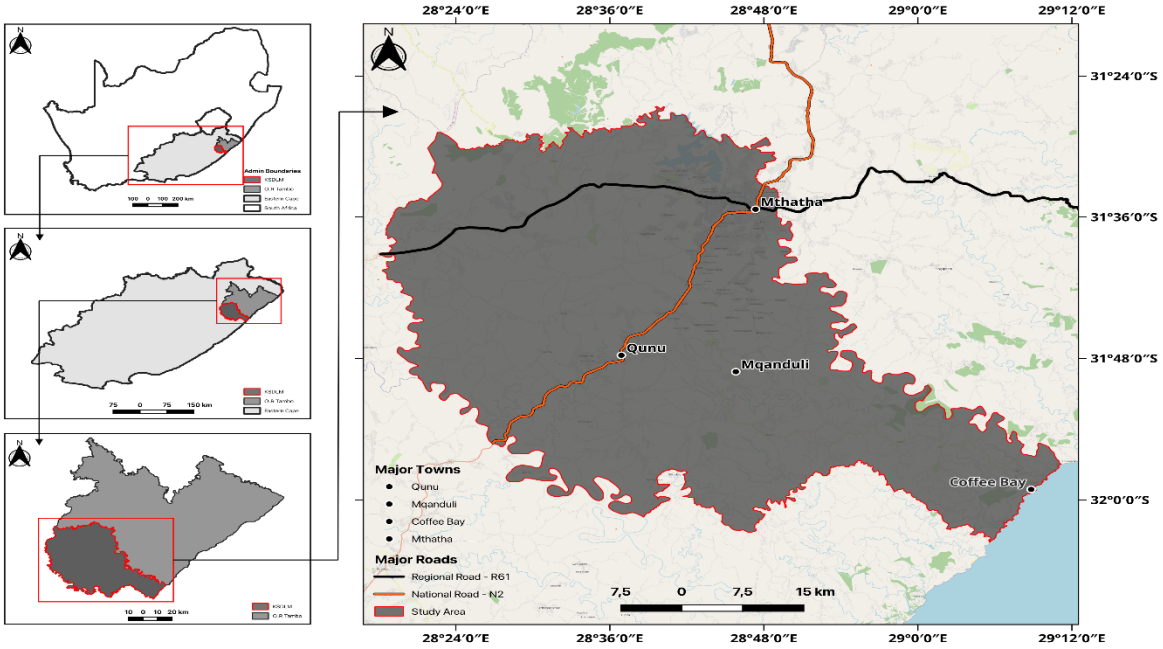


Figure 1. Geographic location of the study area within the Eastern Cape Province, South Africa

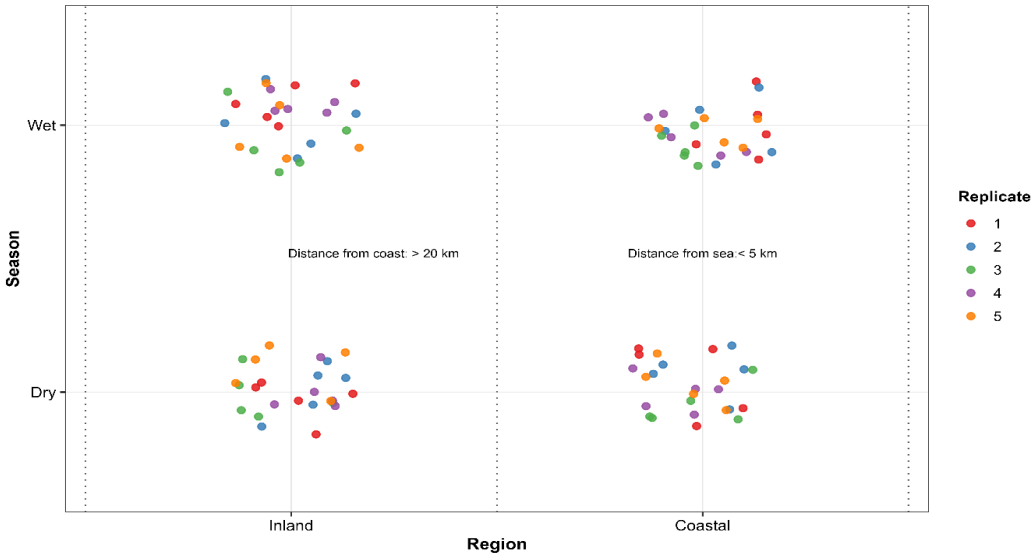


Figure 2. Spatial distribution of springs and seasonal measurement schedule in King Sabata Dalindyebo Local Municipality

successive samples to minimise cross-contamination. The apparatus was also sterilised between successive water samples to reduce the possibility of cross-contamination. Individually packaged sterile membrane filters with a pore size of 0.45 µm, sterile diluent water, and flame-sterilised forceps were used throughout the filtration procedure. The membrane filtration funnels were sterilised by autoclaving before microbial analysis.

For samples that could be enumerated directly, 100 mL of water was aseptically measured and transferred into the sterile filtration funnel. A sterile 0.45-µm membrane was placed on the filtration base using flame-sterilised forceps,

after which the sample was drawn through the membrane under vacuum. The funnel was subsequently rinsed with 20–30 mL of sterile diluent water, and the rinse was drawn through the membrane to facilitate the recovery of microorganisms remaining on the funnel surfaces. The forceps were flame-sterilised again before the membrane was removed from the filtration apparatus.

Where high microbial concentrations prevented reliable direct enumeration, the sample was diluted using sterile diluent water. The dilution factor was recorded and incorporated into the final calculation of microbial concentration.

Following filtration, each membrane was placed onto Brilliance *E. coli*/Coliform Selective Agar (Thermo Scientific, United States), ensuring complete contact between the membrane and the agar and preventing air bubbles from forming. The Petri dishes were covered, inverted, placed in sealed plastic bags, and incubated at 35 ± 0.5 °C for 24 hours to facilitate the recovery of *E. coli* and other coliforms.[31]

After incubation, colonies were enumerated using an illuminated digital colony counter. Based on the chromogenic characteristics of the selective medium, purple colonies were recorded as *E. coli*, while pink colonies were recorded as other coliforms. No additional biochemical or molecular confirmation of the isolates was performed.

Microbial concentrations were calculated from the observed colony count, dilution factor, and volume filtered and expressed as colony-forming units per 100 mL (CFU/100 mL) using **Equation 2**:

$$CFU\ /100\ mL = \frac{Colony\ count \times Dilution\ factor \times 100}{Volume\ filtered\ (mL)} \quad (2)$$

For an undiluted 100-mL sample, the dilution factor was 1. For diluted samples, the observed colony count was multiplied by the corresponding dilution factor.

2.5. Quality assurance and quality control

Sterile sample containers, individually packaged sterile membranes, and sterile diluent water were used to minimise procedural contamination. The reusable filtration apparatus was sterilised before the commencement of analysis and between successive samples. Forceps were flame-sterilised before placing and removing each membrane, and the funnel was rinsed with 20–30 mL of sterile diluent water after filtration. Samples were maintained below 10 °C during transportation and analysed within four hours of collection. Incubation temperature and duration were controlled at 35 ± 0.5 °C for 24 hours.[32,33]

Field blanks and filtration blanks were not included in the analytical procedure. Consequently, contamination that may have been introduced during field collection, transportation, or filtration could not be independently assessed.

2.6. Statistical analysis

Statistical analyses were conducted using R (R Foundation for Statistical Computing, Austria), a programming language and software environment for statistical computing and graphics. Descriptive statistics were calculated for *E. coli* and other-coliform concentrations according to season and region. Because the distributions were skewed, medians, first and third quartiles, interquartile ranges, and minimum–maximum values were reported together with means and standard deviations. The number of observations reported for each group represents repeated sampling observations rather than independent springs.

Separate negative binomial generalised linear mixed-effects models were fitted for *E. coli* and other coliform counts using using glmmTMB function from the glmmTMB package (version 1.1.14). The models were fitted by maximum-likelihood estimation using the Laplace approximation. A negative-binomial distribution with a logarithmic link function was selected to accommodate overdispersion in the count data. Region and season were included simultaneously as fixed effects, while the permanent identity of each of the ten physical springs was included as a random intercept to account for correlation among repeated observations collected from the same spring.

Model coefficients were exponentiated and reported as incidence rate ratios (IRRs), with 95% confidence intervals (CIs) and *p*-values. The dry season and coastal region were used as the reference categories. An IRR below 1 indicated a lower expected microbial concentration relative to the reference category, whereas an IRR above 1 indicated a higher expected concentration. Statistical significance was evaluated at $p < 0.05$.

Microbial concentrations were $\log_{10}(x + 1)$-transformed for graphical presentation in the boxplots only. All negative binomial mixed-effects models were fitted to the untransformed microbial counts.

3. Results

3.1. Descriptive statistics and microbial distributions

3.1.1. Descriptive statistics of microbial concentrations

Descriptive microbial concentrations varied according to season and region (Table 1). Median *E. coli* concentrations

Table 1. Descriptive statistics of E. *coli* and other coliform concentrations in untreated springs by season and region

Microbial parameter	Factor	Group	Observations (*n*)	Mean ± SD (CFU/100 mL)	Median (Q1–Q3) (CFU/100 mL)	IQR (CFU/100 mL)	Minimum–maximum (CFU/100 mL)
E. coli	Season	Dry	50	250.88 ± 118.10	277.5 (167.5–337.5)	170.00	20–470
E. coli	Season	Wet	50	151.36 ± 107.05	114.5 (56.5–269.5)	213.00	4–320
E. coli	Region	Coastal	50	162.72 ± 99.26	170.0 (65.0–235.0)	170.00	4–320
E. coli	Region	Inland	50	239.52 ± 132.66	282.5 (107.75–337.5)	229.75	6–470
Other coliforms	Season	Dry	50	471.56 ± 52.03	467.5 (420.0–518.75)	98.75	400–560
Other coliforms	Season	Wet	50	394.64 ± 379.35	326.0 (313.0–377.25)	64.25	277–3,012
Other coliforms	Region	Coastal	50	383.64 ± 49.20	405.0 (345.25–420.0)	74.75	277–450
Other coliforms	Region	Inland	50	482.56 ± 377.12	487.5 (325.0–520.0)	195.00	298–3,012

Abbreviations: IQR: Interquartile range; SD: Standard deviation.

were higher during the dry season than during the wet season, at 277.5 and 114.5 CFU/100 mL, respectively. Inland springs also showed a higher median *E. coli* concentration than coastal springs, at 282.5 and 170.0 CFU/100 mL, respectively. Median other-coliform concentrations were higher during the dry season than during the wet season, at 467.5 and 326.0 CFU/100 mL, respectively, and higher in inland than coastal springs, at 487.5 and 405.0 CFU/100 mL, respectively. A maximum other-coliform concentration of 3,012 CFU/100 mL was recorded in the wet-season inland group and was retained as a valid dilution-adjusted observation.

Values represent dilution-adjusted concentrations on the original measurement scale. The number of observations (*n*) does not represent the number of independent springs. The regional groups contained 5 springs each, while both seasonal groups contained observations from all 10 springs. Each spring was sampled 5 times per season, yielding 10 observations per spring across the 2 seasons. Because measurements were repeated within springs, inferential comparisons were conducted using negative-binomial mixed-effects models with spring included as a random intercept.

3.1.2. Distribution of microbial concentrations by season and region

The distributions of *E. coli* and other-coliform concentrations by season and region are presented in Figure 3. The boxplots provide a graphical summary of the unadjusted microbial distributions and should be interpreted alongside the mixed-effects model results presented in Section 3.2.

3.2. Mixed-effects model results

After adjustment for region and repeated observations within springs, expected *E. coli* concentrations were 38.6% lower during the wet season than during the dry season (IRR = 0.614, 95% CI: 0.468–0.805, $p < 0.001$; Table 2). Although the observed median was higher in inland springs, the adjusted regional effect was not statistically significant (IRR = 1.346, 95% CI: 0.912–1.987, $p = 0.135$).

Expected other-coliform concentrations were 19.3% lower during the wet season than during the dry season after adjustment for region and repeated observations within springs (IRR = 0.807, 95% CI: 0.720–0.905, $p < 0.001$). Inland springs had expected other-coliform concentrations 23.4% higher than coastal springs after adjustment for season and repeated measurements (IRR = 1.234, 95% CI: 1.019–1.494, $p = 0.031$).

3.2.1. Seasonal effects on microbial concentrations

Seasonal variation was significantly associated with both microbial indicators after adjustment for region and repeated observations within individual springs. Expected *E. coli* concentrations were 38.6% lower during the wet season than during the dry season (IRR = 0.614, 95%

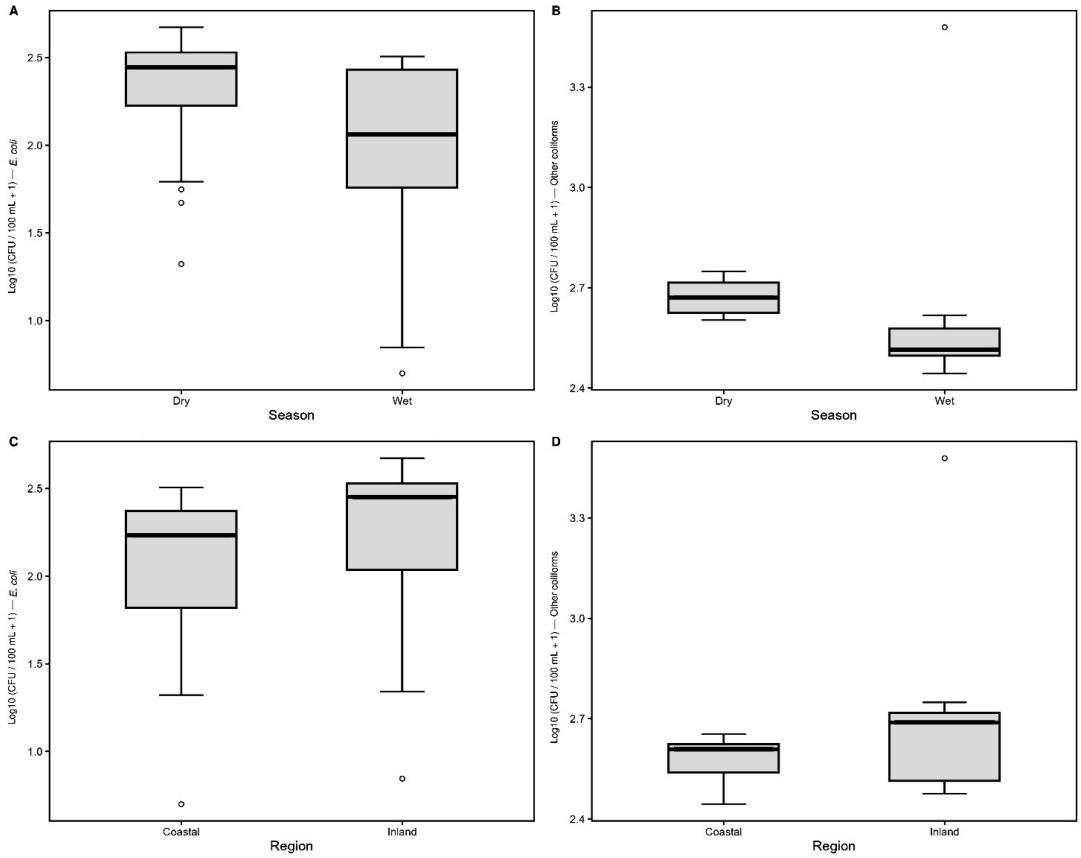


Figure 3. Distribution of microbial concentrations in untreated springs according to season and region: (A) *E. coli* by season, (B) other coliforms by season, (C) *E. coli* by region, and (D) other coliforms by region. For graphical presentation, microbial concentrations (CFU/100 mL) were transformed as $\log_{10}(x + 1)$, where x represents the numerical concentration value. Boxes represent the interquartile range, horizontal lines represent medians, whiskers extend to observations within 1.5 times the interquartile range, and circles represent observations beyond the whiskers. The other-coliform concentration of 3,012 CFU/100 mL was retained as a valid dilution-adjusted observation.

Table 2. Negative binomial mixed-effects model estimates for the effects of season and region on microbial concentrations

Outcome	Predictor	IRR	95% CI	Percentage difference	*p*-value
E. coli	Wet season versus dry season	0.614	0.468–0.805	38.6% lower	<0.001
E. coli	Inland versus coastal	1.346	0.912–1.987	34.6% higher	0.135
Other coliforms	Wet season versus dry season	0.807	0.720–0.905	19.3% lower	<0.001
Other coliforms	Inland versus coastal	1.234	1.019–1.494	23.4% higher	0.031

Notes: Dry season and coastal region were the reference categories. Models included season and region as fixed effects and spring as a random intercept to account for repeated observations from the same physical springs. An IRR below 1 indicates a lower expected microbial concentration relative to the reference group, whereas an IRR above 1 indicates a higher expected concentration. Statistical significance was evaluated at $p < 0.05$.
Abbreviations: CI: Confidence interval; IRR: Incidence rate ratio.

CI: 0.468–0.805, $p < 0.001$). Similarly, expected other-coliform concentrations were 19.3% lower during the wet season than during the dry season (IRR = 0.807, 95% CI: 0.720–0.905, $p < 0.001$). These results demonstrate a consistent seasonal pattern, with higher adjusted microbial concentrations during the dry season for both indicators.

3.2.2. Regional effects on microbial concentrations

The adjusted regional effects differed between the two microbial indicators. Inland springs had an estimated *E. coli* concentration 34.6% higher than coastal springs; however, the 95% confidence interval included 1, and the difference was not statistically significant (IRR = 1.346, 95% CI: 0.912–1.987, $p = 0.135$). In contrast, expected other-coliform concentrations were 23.4% higher in inland springs than in coastal springs, and this regional difference was statistically significant (IRR = 1.234, 95% CI: 1.019–1.494, $p = 0.031$). Therefore, the mixed-effects analysis supported a regional difference in other-coliform concentrations but not in *E. coli* concentrations.

3.3. Microbiological compliance and public-health relevance

E. coli was detected in all 100 spring-water samples, with concentrations ranging from 4 to 470 CFU/100 mL. The South African National Standard (SANS) 241-1:2015 requires *E. coli* to be undetectable in 100 mL of drinking water. Consequently, none of the analysed spring-water samples complied with the SANS microbiological requirement for *E. coli*. Its consistent detection indicates faecal contamination and suggests the possible presence of enteric pathogens.

4. Discussion

4.1 Seasonal variation in microbial concentrations

Season significantly influenced both microbial indicators after accounting for repeated observations within the same springs. *E. coli* concentrations were 38.6% lower in the wet season than in the dry season (IRR = 0.614, 95% CI: 0.468–0.805, $p < 0.001$). Similarly, other-coliform concentrations were 19.3% lower during the wet season (IRR = 0.807, 95% CI: 0.720–0.905, $p < 0.001$). These adjusted findings were consistent with the descriptive results, which showed higher median concentrations during the dry season for both *E. coli* and other coliforms.

The higher dry-season concentrations may reflect reduced spring discharge and a corresponding decrease in dilution capacity. Under low-flow conditions, microorganisms entering or persisting in a spring are distributed within a smaller volume of water, potentially increasing their measured concentrations. Wright[34] similarly reported pronounced seasonal variation in natural drinking-water sources in Sierra Leone, including springs and wells, where faecal-indicator counts increased progressively during the dry season and declined following the onset of rainfall. Dilution associated with increased wet-season water availability was proposed as an important controlling process.

Greater dependence on springs during dry periods may provide an additional mechanism. When alternative water supplies become limited, more frequent water collection and increased livestock use may physically disturb sediments in and around spring outlets. Sediments can act as reservoirs for faecal indicator bacteria, which may subsequently be returned to the water column upon disturbance. In the Apies River in South Africa, experimental disturbance of riverbed sediments, including disturbance caused by cattle crossing the river, increased water-column *E. coli* concentrations by approximately 7.9–35.8 times their initial values.[35] Although that investigation was conducted in a river rather than a spring, it demonstrates a plausible process through which human or animal disturbance could temporarily elevate microbial concentrations in sediment-containing water sources.

Seasonal contamination patterns are not uniform across different hydrogeological and climatic settings. Maželienė *et al.*[26], for example, found that Lithuanian springs experienced their highest microbial contamination during autumn and winter. Their findings demonstrate that the microbiological response of springs may depend on local rainfall patterns, groundwater recharge, surrounding land use, and the degree of protection at individual spring sites. Therefore, seasonal categories alone may not fully explain microbial variability without corresponding measurements of rainfall, recharge, and spring discharge.

Contrasting seasonal patterns have also been reported in systems where rainfall mobilises contaminants. Kumpel *et al.*[36] observed a higher occurrence of thermotolerant coliform contamination during the rainy season in water sources in Port Harcourt, Nigeria. Rainfall can wash faecal material from soils, livestock areas, and poorly managed sanitation facilities towards water sources, while infiltration can transport contaminants into shallow groundwater. Karst springs may respond particularly rapidly to precipitation because preferential flow pathways can connect the land surface to the aquifer. Savio *et al.*[37] observed increases in *E. coli* and other faecal indicators during the initial phase of a high-discharge event in an alpine karst spring, although the microbial response was not reproduced during a subsequent discharge event. This variability illustrates that rainfall does not invariably produce the same microbial response, even within a single

spring system.

The lower overall wet-season concentrations recorded in the present study may therefore indicate that dilution and flushing exceeded the microbial inputs mobilised by rainfall during the sampling period. Nevertheless, the wet-season other-coliform dataset included a concentration of 3,012 CFU/100 mL. This valid dilution-adjusted observation demonstrates that an individual spring could still experience a substantial contamination event during the wet season, despite the lower overall adjusted seasonal concentration. It also emphasises that average seasonal effects should not be interpreted as meaning that every wet-season sample has better microbiological quality.

The seasonal pattern for other coliforms should also be interpreted differently from that of *E. coli*. Whereas *E. coli* is a more specific indicator of faecal contamination, other coliforms can originate from vegetation, soil, sediments, and aquatic environments. Their higher dry-season concentrations may therefore reflect both contamination inputs and environmental processes that favour their persistence or resuspension. The observed reduction in other coliforms during the wet season cannot, by itself, be interpreted as evidence of reduced faecal contamination.

Rainfall, spring discharge, sediment characteristics, livestock access, and the intensity of water-collection activities were not quantitatively measured in this study. The proposed dilution, contaminant transport, and sediment resuspension processes therefore remain plausible mechanisms rather than demonstrated causes of the observed seasonal differences. Future research should combine repeated microbial enumeration with rainfall records, spring discharge measurements, sediment analyses, and structured sanitary surveys to determine the mechanisms controlling seasonal contamination in these springs.

4.2. Regional variation in microbial concentrations

Regional effects differed between the two microbial indicators. After adjustment for season and repeated observations within individual springs, expected other-coliform concentrations were 23.4% higher in inland springs than in coastal springs (IRR = 1.234, 95% CI: 1.019–1.494, $p = 0.031$). In contrast, although expected *E. coli* concentrations were 34.6% higher in inland springs, the adjusted regional effect was not statistically significant (IRR = 1.346, 95% CI: 0.912–1.987, $p = 0.135$). These results indicate that an inland–coastal location was associated with other coliform concentrations but did not adequately differentiate *E. coli* contamination.

The contrasting regional responses may partly reflect differences in the environmental specificity of the two indicators. Coliform bacteria other than *E. coli* include organisms of both faecal and environmental origin and may occur naturally in soil, water, vegetation, and decomposing organic material.[38,39] Genera within the broader coliform group, including *Klebsiella*, *Enterobacter*, and *Citrobacter*, may originate from faecal or non-faecal sources and can persist outside the intestine.[38,39] By contrast, *E. coli* is considered a more specific indicator of faecal contamination because of its stronger association with the intestinal contents of humans and other warm-blooded animals,[7] although some strains can persist or become naturalised in environmental reservoirs.[40,41] The two indicators should therefore not necessarily exhibit identical regional patterns.

Soil and sediment reservoirs may be particularly relevant to the spatial distribution of other coliforms. Sediments, bank soils, and associated organic material can retain faecal indicator bacteria and release them into the water column when disturbed or remobilised.[42] Consequently, the higher other-coliform concentrations observed in inland springs may reflect differences in surrounding soils, vegetation, organic matter, sediment accumulation, or disturbance at individual collection points. Nevertheless, these environmental reservoirs were not measured in the present study, and their contribution to the regional difference cannot be confirmed.

Differences in source protection and surrounding sanitary conditions provide another plausible explanation for variation among springs. Studies of rural drinking-water sources generally report lower faecal-indicator concentrations in protected than in unprotected springs, although protection does not guarantee microbiological safety.[9,14] For example, substantially higher *E. coli* concentrations have been reported in unprotected than protected springs in Lesotho. Inadequate fencing, animal access, and visible animal waste near water sources have also been associated with increased microbial contamination.[43,44] Such hazards operate primarily at the level of individual springs and may occur in both inland and coastal settings. However, source protection and sanitary hazards were not consistently quantified for every spring and sampling event in the present study; therefore, they cannot be identified as the cause of the higher inland other-coliform concentrations.

The absence of a significant adjusted regional effect for *E. coli* suggests that broad inland–coastal classification alone did not explain the distribution of faecal contamination. Although the unadjusted median was higher inland, the regional difference was no longer statistically significant after accounting for season and repeated observations within springs. This finding suggests

considerable heterogeneity among individual springs rather than a uniform regional pattern. Maželienė *et al.*[26] similarly documented marked differences in microbial quality among individual springs within a relatively limited geographical setting, with contamination varying according to local exposure and source conditions. Research in other water environments has also shown that factors associated with faecal-indicator concentrations can vary among sites and across spatial scales.[45,46] Although the latter studies examined surface waters rather than springs, they support the broader interpretation that relationships between environmental conditions and microbial water quality are context- and site-specific.

Hydrogeological conditions may also contribute to variability among individual springs. Shallow or unconfined groundwater systems can be vulnerable to surface-derived contamination because short transport distances and preferential flow pathways may allow microorganisms to reach groundwater before substantial natural attenuation occurs.[47] However, aquifer depth, confinement, fracture connectivity, and groundwater residence time were not determined for the sampled springs. It is therefore not possible to establish whether hydrogeological differences contributed to the observed inland–coastal pattern. Evidence from engineered water-distribution systems concerning stagnation, chlorine depletion, and biofilm regrowth was not considered directly applicable to these untreated natural springs.

Results of this study show that the regional setting contributed to variation in other coliform concentrations but was insufficient to explain *E. coli* contamination. Local environmental reservoirs, source protection, sanitary conditions, and hydrogeological characteristics may contribute to differences among individual springs, but their relative importance was not tested. Future studies should combine repeated microbial monitoring with spring-specific assessments of protection status, sanitary hazards, surrounding land use, sediment characteristics, and hydrogeological connectivity to determine the mechanisms underlying spatial variation.

4.3. Microbiological safety and public-health implications

E. coli was detected in all 100 spring-water samples, at concentrations ranging from 4 to 470 CFU/100 mL. SANS 241-1:2015 requires *E. coli* to be undetectable in 100 mL of drinking water. Consequently, none of the analysed samples complied with the SANS microbiological requirement for *E. coli*, irrespective of season or region. The universal detection of *E. coli* demonstrates that microbiological non-compliance was not restricted to a particular subset of the sampled springs.

The presence of *E. coli* is widely interpreted as evidence of faecal contamination and indicates that water may have been exposed to human or animal waste. Its detection also signals the possible presence of enteric pathogens transported through similar contamination pathways.[7,8] However, specific bacterial, viral, or protozoan pathogens were not investigated in the present study. The results therefore demonstrate faecal contamination and a potential health risk.

The surrounding communities rely on the investigated springs as untreated water sources, making the persistent microbiological non-compliance particularly concerning. Consumption of this water without treatment may expose users to faecally contaminated water; therefore, the sampled spring water should not be considered suitable for direct consumption. The finding remains important even though the *E. coli* concentration was lower during the wet season, because every wet- and dry-season sample exceeded the non-detection requirement. Lower relative concentrations during one season consequently did not indicate microbiological safety.

Reducing exposure requires a combination of source-level and household-level measures. Spring outlets should be protected from direct animal access, uncontrolled surface drainage, and disturbance around collection points through context-appropriate measures such as fencing, diversion of runoff, and maintenance of collection structures. Protection alone, however, cannot guarantee microbiological safety, and routine microbial monitoring remains necessary,[9,14] Where reliably treated supplies are unavailable, households should apply an effective treatment method before consumption, such as boiling, correctly dosed chlorination, or an appropriately maintained filtration and disinfection system. Treatment interventions should be accompanied by safe collection, transport, and covered storage to reduce the possibility of recontamination.

4.4. Methodological limitations and future research

First, the study included 10 springs within a single municipality. Although repeated sampling generated 100 observations and enabled seasonal and regional comparisons, the limited number and geographical distribution of springs restrict the generalisability of the findings beyond the investigated sites. The results should therefore be interpreted as evidence from the sampled inland and coastal springs rather than as representative of all springs in the Eastern Cape or South Africa.

Second, monitoring encompassed selected sampling occasions during one wet and one dry season rather

than continuous or multiyear observation. Microbial concentrations can respond rapidly to individual rainfall events, changes in discharge, and short-term disturbances around spring outlets. The sampling design may therefore not have captured the full temporal variability or extreme contamination events occurring throughout the year.

Third, the environmental mechanisms proposed in Sections 4.1 and 4.2 were not measured quantitatively. Rainfall, spring discharge, sediment characteristics, aquifer depth, hydraulic connectivity, surrounding land use, source-protection condition, and the intensity of human and livestock activity were not incorporated as explanatory variables in the microbial models. Dilution, runoff, sediment resuspension, and sanitary exposure should consequently be understood as plausible mechanisms supported by previous research, rather than as causes established by the present study.

Finally, field blanks and filtration blanks were not included in the documented quality-control procedures. Although sterile sampling containers, cooled transportation, and sterilisation of filtration equipment were used, the absence of these blanks limited the ability to independently detect contamination introduced during field handling or laboratory processing. This limitation should be acknowledged when interpreting the reported concentrations and addressed in subsequent monitoring.

Future research should conduct longer-term, event-based monitoring across more springs and over multiple wet and dry seasons. Microbial sampling should be integrated with rainfall, spring discharge, and physicochemical measurements, structured sanitary inspections, land-use assessment, and hydrogeological characterisation. Future studies should also include field, transport, and filtration blanks; positive and negative laboratory controls; confirmatory identification; pathogen testing; and microbial source tracking. Intervention-focused research should evaluate whether measures such as fencing, runoff diversion, spring-outlet rehabilitation, and household water treatment produce sustained reductions in microbial contamination and exposure risk.

5. Conclusion

This study demonstrated persistent microbial contamination and significant seasonal variation in untreated spring water within King Sabata Dalindyebo Local Municipality. After accounting for region and repeated observations within individual springs, *E. coli* and other coliform concentrations were 38.6% and 19.3% lower during the wet season than during the dry season, respectively. Other coliform concentrations were 23.4% higher in inland springs than in coastal springs, whereas the regional difference in *E. coli* was not statistically significant. These findings indicate that season was consistently associated with both microbial indicators, while inland–coastal location did not uniformly explain their spatial distribution.

E. coli was detected in all 100 samples, and none met the SANS 241-1:2015 requirement for non-detection in 100 mL. Consequently, the sampled spring water should not be considered suitable for direct consumption without appropriate treatment.

The contrasting regional responses of *E. coli* and other coliforms suggest that the indicators may be influenced by different contamination sources and environmental processes. Local conditions at individual springs, including source protection, sanitary hazards, surrounding environmental reservoirs, and hydrogeological connectivity, may contribute to microbial variation. However, these factors were not quantified and cannot be identified as confirmed causes of the observed patterns.

Improving the safety of these water sources requires routine microbial monitoring, protection of spring outlets, control of animal access and surface runoff, and promotion of effective household water treatment and safe storage. Longer-term monitoring incorporating hydrological measurements, sanitary assessments, pathogen identification, and microbial source tracking is required to identify contamination pathways and evaluate the effectiveness of interventions. These measures are essential for reducing exposure among communities that continue to depend on untreated springs for domestic water.

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Conflict of interest

The authors declare that they have no competing interests.

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Availability of data

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

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