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Original Article

Hand hygiene and water quality assessment in schools of Muthanna province, Southern Iraq

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Abstract

Introduction: The severe drought and prolonged heat waves in Iraq have adversely affected the water quality supplied to public facilities. Schools are among the most affected facilities by water scarcity. This work aims to evaluate the level of students' hand hygiene, and the quality of municipal (MW) and drinking water (DW) in some schools of Al-Muthanna Province, Iraq.

Methodology: Between October 2021 and June 2022, a total of 324 water samples from 162 schools and 2,430 hand swabs (HSs) from 1,620 students (1,080 males and 540 females) were collected. Some physicochemical standards of water were assessed besides investigating faecal contamination in water and students' hands using *Escherichia coli* as an indicator.

Results: All MW samples were faecally contaminated with poor standards of pH, turbidity, total dissolved solids, color, and chlorine. Despite the good physicochemical standards of all DW samples, *E. coli* was observed in 12% of samples. Hand hygiene levels dropped by 2.5-fold within a few hours after school entry compared to early-morning levels (before school entry). Male students were 1.5- and 1.7-fold more prone to hand contamination than female students either inside or outside school, respectively. An increasing chlorine tolerance by *E. coli* was observed in water samples with turbidity > 5 NTU and pH > 8.

Conclusions: The students' hand hygiene level decreases within a few hours of entering school, particularly among male students. Residual-free chlorine < 0.5 mg/L with high turbidity and alkalinity in water is insufficient for 100% prevention of *E. coli* contamination.

Key words: *Escherichia coli*; faecal contamination; drinking water; municipal water.

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Introduction

Waterborne pathogens in surrounding water bodies and associated diseases are a significant risk worldwide. Different pathogens, including bacteria, viruses, protozoa, and helminths, were found in water with varying levels of health significance, persistence in water supplies, relative infectivity, and resistance to chlorine [1,2]. Bacterial pathogens, such as *Campylobacter spp, Escherichia coli* (particularly enterohemorrhagic *E. coli* or EHEC), *Burkholderia pseudomallei*, *Legionella pneumophila* and related bacteria, *Non-tuberculous mycobacteria*, *Pseudomonas aeruginosa, Salmonella enterica* serotype Typhi, *Helicobacter pylori*, *Vibrio cholerae*, and *Shigella spp*., are considered among the most common etiological agents of waterborne outbreaks [3]. Water contamination with human and animal faeces can result in several pathogens being introduced into the water supply which can be detected using *E. coli* as the most specific indicator of faecal contamination in water [14,15]. Consumption of contaminated water supplies is associated with a significant percentage of waterborne diseases [4,13]. Children are more vulnerable to environmental hazards compared to adults, making proper water and sanitation in schools particularly important as children spend a significant amount of time at school [5,6]. Since 1990, the United Nations International Children's Emergency Fund (UNICEF) and the World Health Organization (WHO) have implemented a water, sanitation, and hygiene (WASH) program in developing countries. The WASH program has successfully improved access to safe drinking water, sanitation services, and basic handwashing facilities at home worldwide [7]. Given the vital importance of protecting children from the risks of contaminated water, the WASH program has been extended to include schools, where about 570 million children lack basic drinking water services [8]. However, there is limited available data on the achievements of the WASH program in Iraqi schools, and the program is still underfunded [16]. Many reasons have negatively affected the water quality in Iraq, including severe drought and wars, which have led to acute shortages of water for drinking, washing, hygiene, and agriculture [9,10]. Other reasons that have severely affected the water quality and personal hygiene of children include the internal displacement of Iraqi families due to armed conflicts [11,12] and poor health awareness of parents [25].

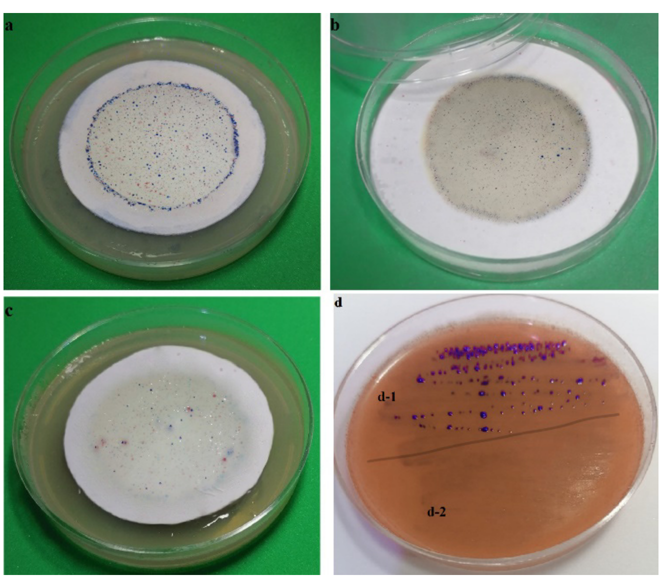
This study aims to evaluate some physicochemical standards of municipal and drinking water provided to schools in AL-Muthanna province southern of Iraq, besides the bacteriological analysis using *E. coli* as an indicator of faecal contamination in water. The study also includes an evaluation of the impact of the school environment on the level of students' hygiene through hand swabbing before/after entering the school and before/after handwashing.

Methodology

Study setting

This study was conducted between October 2021 and June 2022 in Al-Muthanna province, southern of Iraq. Out of 648 schools serving more than 250 thousand students. Six main inclusion criteria have been adopted in the school selection process, namely: no double-shift school, a daytime school (from 8 a.m. until 1 p.m.), no less than 100 students in the school, not a special needs school, an easily accessible school, and a maximum of 30-minute drive from the school to the laboratory. Out of 564 schools, it was planned to visit 282 (50%) schools (172 elementary-, 70 intermediate-, and 40 preparatory schools). The schools were given ascending numbers, then selected randomly using MedCalc program 15.8 (tool: generate random sample).

Figure 1. Escherichia coli (purple colonies) and other coliforms (salmon to red colonies) grown on Chromocult Coliform Agar ES plates. Levels of E. coli contamination in washing water samples as follows: (> 100 cfu/100 mL) (**a**), (50- < 100 cfu/100 mL) (**b**), (< 50 cfu/100 mL) (**c**), no-growth (< 1 cfu/100 mL) (no picture), and direct planting of hand swab (sample **d-1**, control **d-2**).



Sample collection

Prior to water sample collection, swabs were taken from the equipment used for water sample collection and then cultured on blood agar plates. For physicochemical tests, one liter of MW and one-liter of DW were collected and kept in sterilized Duran screw-cap bottles. For *E. coli* screening, MW and DW samples were stored separately in sterile 200 mL containers with 0.5 g sodium thiosulfate to neutralize free and combined chlorine. Hand swabbing was carried out according to the following method: The cotton swab was dipped in 5 mL of normal saline solution, then wiped over the palms, back, fingertips, and fingernails of both hands, and then placed in a screw-capped McCartney bottle containing 5 mL of Amies Transport Agent (Oxoid, UK). In each school, the students were divided into two groups: five students in the control group to collect five HSs before school entry (HSs were collected at the school gate at almost 8 a.m.). Five students in the experimental group collected five HSs before handwashing and five swabs after. HSs of the experimental group were usually collected after 10 a.m. (to ensure sufficient contact time between the students and the school environment). The target students were any students who intended to wash their hands, especially before/after using the toilet or engaging in sports lessons. Samples were kept in tamper-evident bags in a portable freezer and shipped to the lab for bacterial identification within 30 minutes.

Samples test

Swabs were aseptically cultured in Chromocult Coliform Agar ES plates (Merck KGaA, Germany) and incubated at 37 °C for 24 hours. To exclude false-positive results due to unintended bacterial contamination, some agar plates were divided into two parts (sample and control part); the sample part was streaked with HS, and the control part was streaked with normal saline, which had been used to moisturize students' hands. If any bacterial growth (particularly with *E. coli*) occurred in the control part, the agar plate was excluded. The membrane filtration technique was used to assess the *E. Coli* burden in MW and DW samples. The technique involved filtering 100 mL of water sample through a cellulose ester membrane filter (47 mm in diameter with a pore size of 0.45 µm) (Sigma-Aldrich, USA). Then, the membrane was placed on a Chromocult Coliform Agar ES plate and incubated at 37 °C for 24 hours. Purple colonies on the filter membrane were counted and expressed as colony-forming units per 100 mL (CFU/100 mL) (Figure 1). The physicochemical parameters of MW and DW samples included the test of color, turbidity, total dissolved solids (TDS), pH, and chlorine (total, combined, and free chlorine).

Statistical analysis

The continuous and categorical variables were expressed as mean ± standard deviation and percentages, respectively. The t-test for the comparison of two independent means was used to investigate the differences in means. Chi-squared test for trend was used to predict a systematic trend in the proportion of hand hygiene and municipal water contamination. The McNemar test was used to examine the difference in the proportions of hand contamination among students in the experimental group before and after handwashing. The chi-squared test for the comparison of two proportions was used to test the difference in the proportions of students' hand contamination between the control group and the experimental group. All the statistical tests, including odds ratios and 95% confidence intervals, were calculated using MedCalc Version 20.112. A p value ≤ 0.05 was considered statistically significant.

Ethics Statement

This study was conducted after obtaining all written consents from the School Environment and Health Department/General Education Directorate of Al-Muthanna Province. Study details, including procedures and purpose (except the timing of study to ensure that no extraordinary health measures were taken by parents or school administrations), have been declared by schools to inform parents and obtain their written or verbal consents. Before sample collection, it was confirmed that there were no previous objections from school staff or students' parents. All samples were collected under the supervision of at least one school staff member, preferably the science teacher or the headteacher. Female students had special privacy in collecting samples due to the Islamic and social restrictions that prohibit contact with strangers. According to the instructions of Education Directorate, no demographic information was allowed to be collected regarding the standard of living, housing address, housing type (urban/rural), family size, and family type (single or two-parent family).

Results

According to the six inclusion criteria, 84 schools were excluded in the initial survey (before the school visit). After school field visits, 120 schools were excluded: 117 schools were excluded due to the unavailability of public drinking and municipal water samples, and some school administrators and students declined to participate in the study for many reasons: the most important of which was fear of strangers, previous poor research experience, ignorance of health research, and some girls' schools were excluded due to social and religious restrictions (Islamic tradition) that prohibit physical contact with strangers. In addition, three schools whose samples were excluded due to evidence of equipment contamination during the initial sampling phase. A total of 324 water samples (162 MW and 162 DW samples) were collected from 162 schools (91 elementary, 42 intermediate, and 29 preparatory schools), along with 2430 HSs from 1620 student volunteers (1,080 males and 540 females).

Municipal Water MW

The bacteriological analysis showed that all samples were contaminated with *E. coli*, with rates of < 50 CFU/100 mL (41.4%), 50- < 100 CFU/100 mL (41.4%), and > 100 CFU/100 mL (17.2%). The mean values for turbidity, total dissolved solids (TDS), and

Table 1. Mean ± standard deviation of the physicochemical properties of municipal and drinking water.

***E. coli* level**		**Parameter (WHO's preferable levels)**				**Chlorine mg/L**		
Samples type	**CFU/100 mL (number of samples)**	**pH (6.5-8.5)**	**Turbidity (1-5 NTU)**	**Color (< 15 TCU)**	**TDS (<600 mg/L)**	**free**	**combined**	**total**
Municipal Water (MW)	< 50 (67)	7.6 ± 0.45	8.5* ± 2.43	15.3* ± 2.25	935.9* ± 121.1	0.00	1.31 ± 0.45	**
	50- < 100 (67)	8.1 ± 0.43	7.92* ± 2.34	15.6* ± 2.17	878.1* ± 128.7	0.00	1.25 ± 0.47	**
	> 100 (28)	8.7* ± 0.12	7.65* ± 2.6	15.9* ± 2.1	914.6* ± 139.6	0.00	1.22 ± 0.37	**
Drinking water (DW)	Non (142)	8.3 ± 0.59	< 1	0.1	40.9 ± 16.4	0.64	1 ± 0.47	1.65 ± 0.44
	< 50 (20)	8.4 ± 0.56	< 1	0.1	97.3 ± 21.8	0.48	1.29 ± 0.66	1.77 ± 0.41

* the physicochemical levels overstepping WHO's preferable limits; ** equal to combined chlorine; TDS (Total Dissolved Solids); TSS (Total Suspended Solids); TCU (True color unit).

color were higher than the preferable levels of the World Health Organization, in addition to zero residual free chlorine in all samples. The mean pH of > 100 CFU samples was 8.7, significantly higher than the mean pH of < 50 CFU (mean pH = 7.6) and 50- < 100 CFU (mean pH = 8.1) with mean differences of 1.1 (95% CI [0.93-1.3], $p < 0.001$) and 0.6 (95% CI [0.44-0.76], $p < 0.001$), respectively, Table 1. There was no significant mean difference of turbidity, color, and chlorine (combined) except the mean TDS of < 50 CFU samples which was significantly higher than the mean TDS of 50- < 100 CFU at a mean difference of 57.8 (95% CI [15.1-100.5], $p < 0.008$), as shown in Table 1.

Drinking Water DW

The bacteriological analysis showed that 12.3% of the samples were contaminated with *E. coli* at a rate of < 50 CFU/100 mL. The mean pH of samples showed an alkaline tendency with normal levels of turbidity, TDS, and color. The mean values of residual free chlorine were 0.64 and 0.48 in free *E. coli* samples and in < 50 CFU samples, respectively. There was no significant mean difference in pH, turbidity, color, and chlorine (total, combined, and free) between the free *E. coli* samples and < 50 CFU samples except the mean TDS of < 50 CFU samples which were significantly higher than the mean TDS of free *E. coli* sample at mean difference 56.5 (95% CI [48.3-64.5], $p < 0.001$), as shown in Table 1.

Hand Hygiene

The levels of hand contamination showed a significant positive linear trend with increasing contamination with *E. coli* of MW. As the *E. coli* load increased from < 50 CFU to 50- < 100 CFU to > 100 CFU per 100 mL of MW, the hand contamination rate increased from 16.7% to 21.8% and 72%, respectively. As shown in Table 2, the level of hand contamination among total male students in the experimental group was 31%, which was reduced to 25.4% after handwashing, with a significant proportion difference of 5.6% [95% CI 1-11.1], $p = 0.048$. Handwashing had a greater positive effect among total female students in the experimental group compared to males, as it resulted in a significant reduction in hand contamination from 23.3% to 8.6% with a significant proportion difference of 14.7% [95% CI 8.4-21], $p < 0.001$.

It can be also noted that students in the control group have better hand hygiene levels than students of experimental group. The level of hand contamination among students before entering school (control group) was 13.83%, which is significantly lower than the level of hand contamination (28.4%) among students within hours after entering school (experimental group before handwashing) with a significant proportion difference of 14.57% [95% CI 10.56-18.55], $p < 0.001$. Moreover, this level of hand contamination (13.83%) was also significantly lower than the level of hand contamination (19.8%) of the experimental group even after handwashing, with a proportion difference of 5.97% [95% CI 2.25-9.69], $p = 0.002$. This makes students inside schools 2.5 [95% CI 1.9-3.2], $p < 0.001$ times more likely to get hand contamination with *E. coli* compared to students before entering schools. Male students are more susceptible to faecal hand contamination than female students, whether inside or outside schools, with odds ratios of 1.5 [95% CI 1.05-2.06], p = 0,024, and 1.7 (95% CI [1.1-2.7], $p = 0.027$), respectively. Table 3 shows no significant difference in the odds of hand contamination between female students of different ages. It seems that the older the students, the lower the level of hand contamination, which appears to be more evident among the students in the experimental group than in the control group.

Table 2. Number and percentages of *Escherichia Coli* hand contamination in the experimental and control groups.

Gender	School grade	School number	Total number of students	Bacterial contamination[a] positive/total (%)		Bacterial contamination[d] positive/total (%)
				Before[b]	After[c]	
	Elementary	56	560	106/280 (38)	91/280 (32.5)	48/280 (17)
Male	Intermediate	31	310	46/155 (29.7)	34/155 (21.9)	25/155 (16)
	Preparatory	21	210	15/105 (14.3)	12/105 (11.4)	12/105 (11.4)
Total Male		108	1080	167/540 (31)	137/540 (25.4)	85/540 (15.7)
	Elementary	35	350	43/175 (24.6)	17/175 (9.7)	19/175 (10.86)
Female	Intermediate	11	110	12/55 (21.8)	5/55 (9.1)	5/55 (9.1)
	Preparatory	8	80	8/40 (20)	1/40 (2.5)	3/40 (7.5)
Total Female		54	540	63/270 (23.3)	23/270 (8.6)	27/270 (10)
Total		162	1620	230/810 (28.4)	160/810 (19.8)	112/810 (13.83)

[a] Experimental group; [b] Before handwashing; [c] After handwashing; [d] Control group.

Discussion

In Iraq, water for human usage is available in two main ways: the first is the piped municipal water (MW), which is often used for washing, cleaning, or drinking, especially in remote and poor neighborhoods. The second is low-priced drinking water (DW), which is purchased mostly in 20-liter cans from non-governmental desalination plants. There are other sources of water, such as bottled water and well water, which are almost negligible in terms of human consumption compared to the first two sources. In accordance with the World Health Organization recommendations [32] and to achieve the minimum level of water quality assessment, the current study examined: pH, turbidity, total dissolved solids, color, chlorine levels, and fecal contamination analysis using *E. coli* as an indicator. As shown in Table 1, all physicochemical parameters of MW samples were substandard regardless of the levels of bacterial contamination, either < 50 CFU, 50- < 100 CFU, or > 100 CFU, except for the pH level, which tended to be more alkaline in > 100 CFU water samples. The current values of the physicochemical properties of MW are much worse than those obtained by Al-Hassen and Al-Badri [17], Al-Dulaimi and Younes [18], Hassan and Al-Azzawi [19], and Al-Azawi [20]. The residual free chlorine level in all MW samples was 0.00 mg/L, which may indicate insufficient chlorine dose (insufficient to meet chlorine demand), chlorine decomposition by reaction with dissolved metal from corroded pipes, water age (older water contains less chlorine), or reaction with microbial biofilms [35]. The WHO recommends that a minimum residual chlorine concentration of 0.2 mg/L should be maintained in the tap water to the point of consumption in order to adequately inhibit harmful microorganisms [32]. However, despite the acceptable physicochemical properties of DW and a mean residual free chlorine content of approx. 0.48 mg/L, bacterial contamination with *E. coli* at levels < 50 CFU/100 mL was detected in about 12% of the samples. Any detectable levels of *E. coli* in drinking water pose a healthy challenge, even at low levels. Some strains, such as Shiga-like toxin-producing *E. coli* (STEC O157:H7), have a low infectious dose of fewer than 100 CFU/100mL [1]. Both MW and DW showed a mean alkaline pH higher than 8. Chlorine is less effective in alkaline pH > 8, and higher chlorine concentrations with longer contact time are required to achieve effective disinfection [32]. Effective water disinfection by chlorination depends on other factors, including the level of turbidity. The WHO (2017) recommends reducing the water turbidity to < 1 NTU where applicable prior to chlorination. A turbidity above 5 NTU requires a higher dose of chlorine or longer contact time to inactivate waterborne pathogens [32].

Despite the low persistent of *E. coli* to disinfection by chlorine, as reported by WHO [1], Owoseni *et al*. mentioned that a free chlorine of 0.5 mg/L may be insufficient to remove some strains of *E. coli* from water [28]. Adefisoye and Olaniran referred to many emerging observations of low disinfection efficacy of chlorine at the recommended dose [27]. Al-Berfkani *et al.* reported that many strains of *Aeromonas hydrophila, Micrococcus varians,* and *Staphylococcus aureus* have been isolated from chlorinated Iraqi municipal water with high resistance to chlorine and antibiotics [26]. Onyango *et al*. found that the initial high *E. coli* burden in the raw water could be the reason for the resistance of some bacteria to the chlorination process [21]. Furthermore, untreated hospital wastewater is characterized by numerous contaminants, such as pharmaceutically active compounds, antibiotic-resistant microorganisms, persistent viruses, and antibiotic-resistant genes [23,34]. This may also be one of the reasons that explain the bacterial contamination in most water samples of the current study, since the raw water entering the desalination plants in Iraq has

Table 3. Odds ratios of *Escherichia Coli* hand contamination among male and female students of different school grades.

Gender	School grade	Bacterial contamination[a] Odds ratio [95% CI], *p* value				Bacterial contamination[d] Odds ratio [95% CI], *p* value	
		Before[b]		After[c]			
Male	Elementary	*		*		*	
	Intermediate	0.7 [0.5-1], 0.087	*	0.58 [0.37-0.9], 0.02**	*	0.93 [0.55-1.58], 0.79	*
	Preparatory	0.27 [0.2-0.5], < 0.001**	0.4 [0.21-0.75], 0.005**	0.27 [0.14-0.5], 0.001**	0.46 [0.23-0.94], 0.03**	0.62 [0.32-1.23], 0.17	0.67 [0.3-1.4], 0.29
Female	Elementary	*		*		*	
	Intermediate	0.86 [0.41-1.77], 0.68	*	0.93 [0.33-2.65], 0.89	*	0.82 [0.29-2.3], 0.71	*
	Preparatory	0.77 [0.33-1.79], 0.54	0.89 [0.33-2.45], 0.83	0.24 [0.03-1.8], 0.17	0.26 [0.03-2.29], 0.22	0.67 [0.19-2.4], 0.53	0.81 [0.18-3.6], 0.78

*reference category; **significant *p* value; [a] Experimental group; [b] Before hand washing; [c] After hand washing; [d] Control group.

very poor quality, as wastewater from factories, hospitals, and households besides tons of municipal solid waste, are dumped into rivers without pre-treatment [22,31,33]. Based on the above, there are two reasonable causes that can explain the low effectiveness of chlorine in drinking water. The most acceptable of these is that *E. coli* has developed a tolerance to chlorine, and the second is that alkaline water reduces the effectiveness of chlorine.

Table 2 shows that students in the control group have better hand hygiene levels than students in the experimental group, either before or after handwashing. Furthermore, poor hand hygiene was found in schools with > 100 CFU/100mL of MW samples, supporting the claim that some schools in AL-Muthanna province have become potential sources of bacterial contamination. This claim can be generalized to the most schools in the rest of Iraqi cities, with a similar, if not worse, level of infrastructure compared to the current study, particularly in some densely populated cities like Basra, Thi-Qar, and Maysan. Despite all MW samples being contaminated with *E. coli* at different levels, it seems to have reduced the levels of hand contamination by 5.6% and 14.7% among male and female students, respectively.

Besides the contaminated municipal water, the transmission of bacterial contamination from one student to another is an additional risk factor that reduces hand hygiene levels. Where at least 1 log10 CFU of *E. coli* on the carrier's hands is sufficient to transmit bacterial contamination to another person (host) upon hand contact [30]. Kavitha *et al* found that many potential pathogens (e.g. *E. coli, Enterococcus faecalis, Staphylococcus aureus,* and *Klebsiella spp*) were isolated despite handwashing [24]. Since it seemed that handwashing once or twice during school hours may not be sufficient for keeping hand hygiene, Boehm *et al*. [29] pointed out that the *E. coli* concentration in both hands is increased during some activities, e.g., housework, to 2-3 log CFU within a few minutes even after handwashing. Most of the visited schools lacked handwashing soap, sufficient tap water, and either inadequate or poor handwashing facilities. It was also observed that dozens of students prefer to wash their hands and faces with tap water only. As well as the drinking water was stored in only two ways, the first was in 20-liter jerry cans and the second was in hand-filled water dispensers with loose lids. Based on these observations, it is likely that the bacterial contamination of DW was due to external factors (e.g. contaminated water containers, non-compliance with hygiene conditions during filling of DW) and not due to poor water disinfection processes.

Conclusions

These results suggest that schools have become a potential source of bacterial infection, even for students with good personal hygiene. The odds of hand-faecal contamination increase by 2.5 times inside the school, particularly among male students. It can be concluded that the amount of residual free chlorine of less than 0.5 mg/L is not sufficient for 100% elimination of *E. coli*. Turbidity > 5 NTU and pH > 8 reduce the disinfecting activity of chlorine and increase chlorine tolerance by *E. coli*.

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